PRELIMINARY PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES OF THE FLOWERS OF *STRIGA SENEGALENSIS* (SCROPHULARIACEAE)

BY

UMAR MOHAMMED SANI

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B Sc. (HON.) ABU, ZARIA

A thesis submitted to the Postgraduate School, Ahmadu Bello University, in "Partial"
fulfillment of the requirements for the Degree of MASTER OF SCIENCE IN PHARMACEUTICAL CHEMISTRY

DEPARTMENT OE PHARMACEUTICAL AND MEDICINAL CHEMISTRY
FACULTY OE PHARMACEUTICAL SCIENCES, AHMADU BELLO UNIVERSITY, ZARIA

NOVEMBER, 1997

DECLARATION

This is to certify that I carried out the work in these thesis title preliminary phytochemical and pharmacological studies of the flowers of striga senegalensis (SCRO PHULARIACEAE). In the Department of Pharmaceutical and Medicinal Chemistry Ahmadu Bello University, under the supervision of Dr. M. K. Choudhury (main supervisor) and Professor Abdullahi Mustapha. The work of other investigators is acknowledged and referred to accordingly. I am solemnly declared that no part of this thesis has been submitted elsewhere for a degree.

Um ar Mohammed Sani

CERTIFICATION

This thesis, prepared by UMAR MOHAMMED SANI on Preliminary Phytochemical and Pharmacological Studies of the flower of striga senegalensis [SCROPHULARIACEAE] has met the regulation governing the award of the Degree of Master of Science in the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria and is hereby approved and endorsed for its contribution to scientific knowledge and literary presentation.

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V.C. Agwada
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Prof. J.Y. Olayemi
DEAN POSTGRADUATE
Ahmadu Bello University, Zaria.

DEDICATION

This piece of work is dedicated to ***ALLAH*** Subhanahu Wata'ala who gave me the courage to finish the work against all difficulties.

ACKNOWLEDGEMENT

I wish to, from the depth of my heart, express my deep gratitude to my able major Supervisor Dr. M. K. Choudhury for his excellent supervision of this work. I do not think I will ever forget this kind gentleman.

Professor Abdullahi Mustapha (a Co-supervisor) and Dr. Magaji Garba will remain in my conscious mind for the rest of my life for the support, advice, guidance and assistance in various forms they gave me throughout the course of this work. I must not also forget Professor B. B. Danbatta (Vice Chancellor, Bayero University Kano, BUK) and Dr. Mas"ud Adamu Kazaure for helping me acquire the spectral data (UV, IR, and Mass) and GC Chromatograms of some of my samples from Leeds. Dr. M. M. Idris (HOD Chemistry, BUK) must equally be remembered and thanked for allowing part of this work to be carried out in one of his laboratories. I can never also forget the assistance given me by Dr. Haruna Adamu particularly with regards to some aspects of pharmacological studies of this work. I this very connection Mallam Ibrahim of the Department of Pharmacology and Clinical Pharmacy, ABU, Zaria, must also be thanked.

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ABSTRACT

Phytochemical studies including colour tests and spectral analysis (UV and IR) revealed the possible presence of flavonoid compound(s) having a 5-hydroxyl function in the purified ethanolic extract (E) of the flowers of the plant *Striga senegalensis*. Gas Liquid Chromatography coupled with Mass Spectrometry(GC-MS) of E also indicated the presence of nonane, 2-methyl-1-nitropropane and trimethyl borate (or methyl borate or boric acid trimethyl ester).

Antifertility test has indicated that the crude ethanolic extract (ET) of the flowers has a dose-dependent antiimplantation effect with 80 mg and 40 mg doses suppressing implantation in 80% and 20% of the treated female albino rats, respectively. Both the extracts, ET and E, have shown dose dependent contraction activities on isolated guinea-pig ileum requiring about 15mg of ET and 8.67mg of E to produce comparable response to that produced by 0.05ug of acetylcholine.

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CHAPTER ONE

INTRODUCTION

1.1 Antifertility Agents

The plant kingdom is a rich source of biologically active agents showing various types of pharmacological activities. In 1978 six collaborating centres were set up in different parts of the world to contribute to the collection, testing and identification of antifertility agents from plants identified by an extensive computer analysis of published information (Olaniyi, 1989).

Research on Indian plants with antifertility effects has been exhaustively reviewed (Chaudhury and Haq, 1980; Kamboj and Dhawan, 1982; Sayavati, 1984). Plants such as *Cuminum cyminum Linn, Dolichos biflorus Linn, Hyptis suavealens poit and Trianthema pentandra Linn,* have been repeatedly mentioned in the literature as anti-fertility agents (Kirtika and Basu, 1935; Nadkarni and Nadkarni, 1954; Chopra et al., 1956). The following plants have also been mentioned to have promising antifertility activities: *Sophom occidentalist* L. , *Cajanus cajan* L. Mill, *Pisum sativum* L. , *Abrus precatorius* L. , *Carica papaya* L. , *Hibiscus rosa, Oladenlandia affinis, Sapindus trifoliatus* and *Momordica charantia* (Olaniyi, 1989).

The various parts of the plant *Butea frondosa* (also known as *Butea monosperma*) have been reported to be used in the Ayurvedic system of medicine in India (Nadkarni and Nadkarni, 1954). The classic Ayurvedic text, Vegbhata, however, does not mention the antifertility activity of this

plant (Godbole et al., 1966), although this activity has been alluded to (Kirtika and Basu, 1935). The antifertility effect of this plant in experimental animals has been observed in the crude extracts of its seeds and the purified crude extract of the petals (Hetherington, 1968; Kaleysaraj and Kurup, 1963). The antifertility activity and its mechanism of action of the seed extract of the plant has also been shown (Razdan et al., 1969). When the seeds of this plant were extracted in ethanol for 8 days at room temperature, the residual seed powder was found to be devoid of any activity (Razdan et al., 1969). Some other studies on this plant has also indicated that the ethanolic extract and not the chloroform or the aqueous extracts possessed antifertility activity (Dreisback, 1963). A complete suppression of pregnancy has also been reported in rats receiving 300mg/ kg body weight of the ethanolic extract of the seeds of this plant (Chaudhury and Khanna, 1968).

While screening four Indian indigenous plants, petroleum ether (60-80°C), 95% ethanol and distilled water were used to extract with soxhlet extractor from the seeds of *Cuminum cyaminum* and *Dolichos biflorus* and also from the leaves of *Trianthema pentandra* and *Hyptis suaveolens*, successively (Garg, 1976). The alcoholic and aqueous extracts of the seeds of *Cuminum cyaminum* inhibited implantation in 100% and 80% of female albino rats at the doses of 150mg/kg and 200mg/kg body weight respectively, when administered orally. At the dose of 125mg/kg body weight, the alcoholic extract of *Hyptis suaveolens* was found to inhibit implantation in 100% of the treated rats. The petroleum ether extract of *Cuminum cyaminum* and both the petroleum ether and aqueous extracts of *Hyptis suaveolens* did not exhibit encouraging activity. All the three

extracts of both *Dolichos biflorus* and *Trianthema pentandra* have been found to be devoid of any significant activity even at a dose of 200mg/kg body weight.

The whole plant *Striga lutea* including the roots, stem, leaves and flowers was successively subjected to soxhlet extraction with petroleum ether (60-80° C), chloroform, 95% ethanol and distilled water for 18-20 hours (Hiremath and Rao, 1990 a). Results indicated that both the petroleum ether and chloroform extracts exhibited complete and partial resorption of implants in mice at the doses of 100mg/kg and 50mg/kg body weight, respectively. The crude chloroform extract was also found to have antifertility activity in mice and rats when fed orally post coitum at single dose (Hiremath and Rao, 1990 a).

1.1.1 *Oestrogenic Activity*

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Many oestrogenic steroids and non-steroidal compounds have been tested for their antifertility activity in rabbits and hamsters and a conclution has bee made that any compound with oestrogenic activity may have antifertility activity if taken at a particular time soon after mating (Chang and Yanagimachi, 1965). Another conclusion was also drawn that there was " little doubt that antifertility is due to oestrogenic activity" after testing twelve steroids and fifteen non-steroids for their oestrogenic, antioestrogenic and antifertility activity in the mouse (Emmens, 1965).

The oral contraceptive agents have been successful in controlling fertility and are thus considered to be antifertility drugs (Olaniyi, 1989). The primary therapeutic use of oestrogens as oral contraceptives is that they are

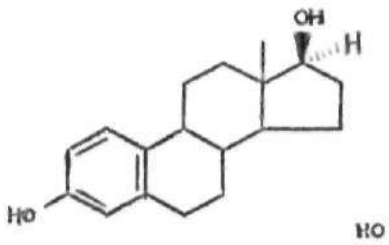
reputed to possess anti-implantation activity (Hiremath and Rao, 1990 b). The antifertility drugs prevent conception in several ways including: expulsion of the ova from the oviduct (Pincus, 1965); disturbing the functional equilibrium between the endogenous oestogens (oestradiol, oestrone and oestriol) and progesterone (Psychoyos, 1966); making the endometrium less suitable for implantation of the fertilized ovum and also inhibiting ovulation by blocking the release of Luteinising Hormone (Trounce, 1985); and changing the character of the mucus in the uterine cervix, becoming viscous and making penetration of sperms to uterus more difficult.

Clinically useful compounds with oestrogen-like activity include mestranol (1), ethinyloestradiol (2), and stilboestrol (3) and those with progestrone-like activity include ethisterone (4), norethisterone (5), lynoestrenol (6) and norethynodrel (7). There are many combinations of oestrogens and progestagens as oral contraceptive agents and many of these formulations contain ethinyloestradiol as the oestrogen and the rest mestranol. The progestagens are all 19-norsteroids.

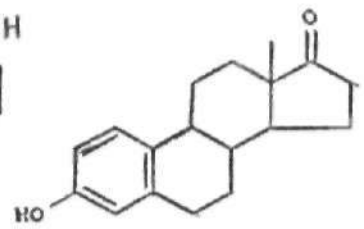
1.1.2 *Isoflavonoids*

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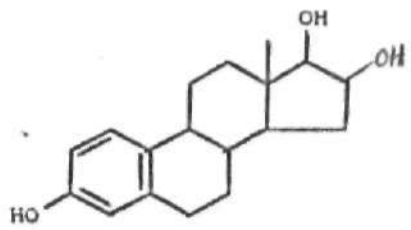
Although much of the research into the development of new oral contraceptives has centred around synthetic modifications of existing steroids, the potential value of plants as sources of new antifertility agents has been recognised. Studies on the new oestrogenic activity of isoflavones were initiated after an unexpected observation of infertility developed in breeding sheep while grazing on pastures of subterranean clover (Bennets



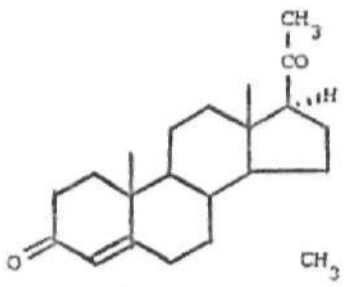
17β - Oestradiol



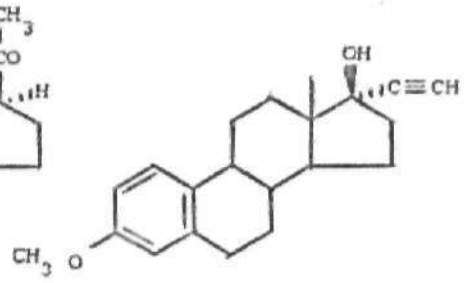
Oestrone



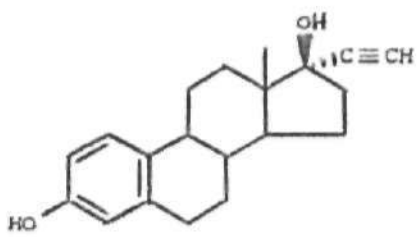
Oestriol



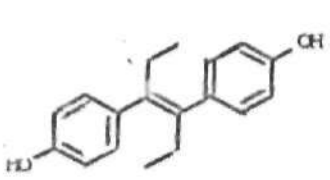
Progestrone



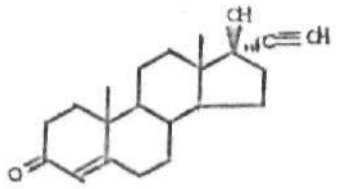
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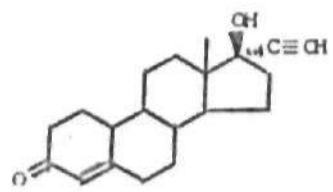
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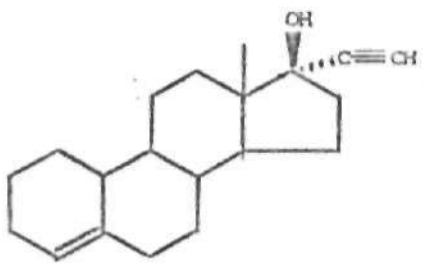
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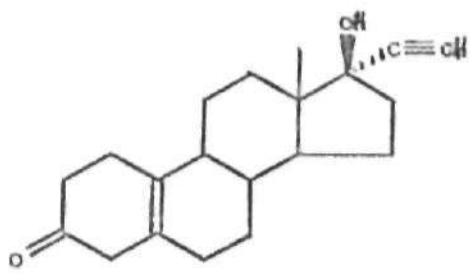
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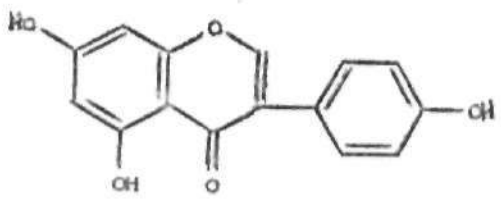


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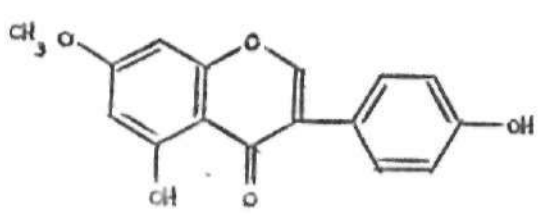
and Underwood, 1949). Similar observations were also made in sheep and rabbits while they were fed on soyabean hay (Hausa: Waken soya) (Sharma et al., 1971).

The oestrogenic activity of the subterranean clover has been attributed to the active principle genistein (8) which has been previously reported to be present in *Genestra tinctoria* and *Soja hispida* (Soyabean) (Sharma et al., 1971). Among the other isoflavones studied for oestrogenic activity include prunetin (9), biochannin-A (10), formononetin (11) and daidzein (12)

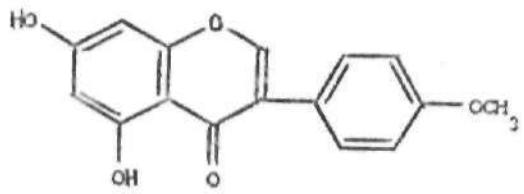
Formononetin (11) (7-hydroxy-4'-methoxyisoflavone) and daidzein (12) (7,4'-dihydroxyisoflavone) were found to be devoid of any oestrogenic actvity while biochannin-A (10) (5,7-dihydroxy-4'-methoxyisoflavone) and prunetin (9) (5,4'-dihydroxy-7-methoxyisoflavone) have been observed to have antifertility activity and this activity has been attributed to the presence of 5-hydroxyl group (Bradbury and White, 1953). It has also been reported that the relative oestrogenic potencies of plant oestrogen are in the order: coumestrol (13)> genistein(8)> biochannin-A (10)> formononetin (11) (Sharma et al., 1971). It has been observed that the isoflavone, irisolone (5,7-dihydroxy-4',6-dimethoxyisoflavone) (14), after oral administration in 3mg dose caused a slight increase in uterine weight while irilone (6,7-methylenedioxy-4'-hydroxyisoflavone) (15) showed no effect even at a dose of 5mg (Sharma et al., 1971). These findings were in support of the Bradbury and White (1953) who claimed that a hydroxy group is essential for oestrogenic activity.



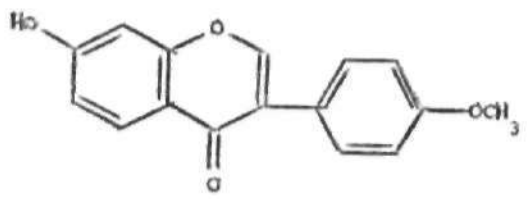
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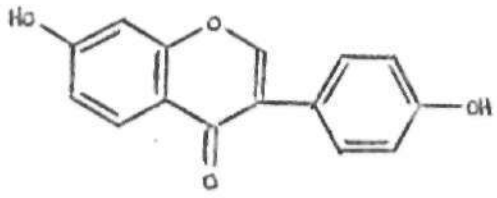
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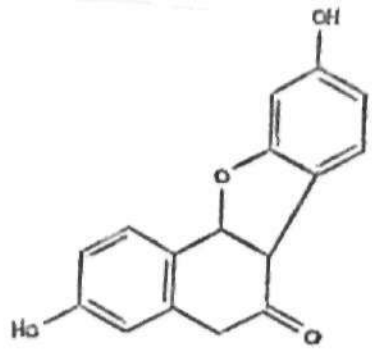
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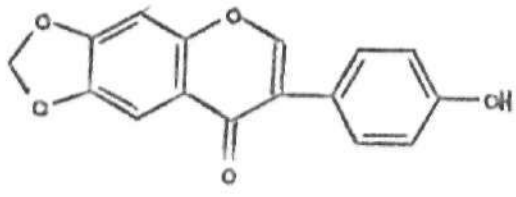
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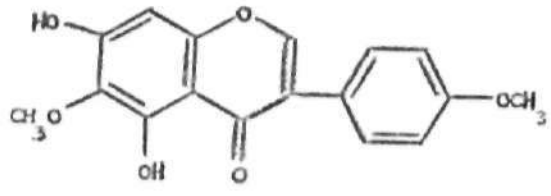
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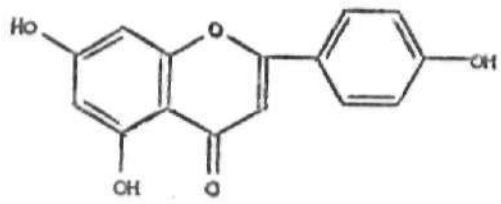
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1.1.3 *Flavonoids*

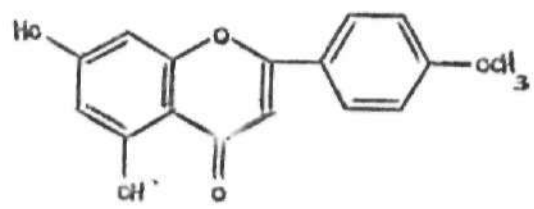
The flavone butrin isolated from the petals of the plant *Butea frondosa* has been found to have clear antifertility activity in rats (Razdan et al., 1969). Six flavones were isolated and characterized from the whole plant, *Striga asiatica* a species belonging to the family *Scrophulariaceae* (Nakanishi et al., 1985). These flavones were: apigenin (5,7,4'-trihydroxyflavone) (16), acacetin (5,7-dihydroxy-4'-methoxyflavone) (17), acacetin 7-methyl ether (18), luteolin (5,7,3',4'-tetrahydroxyflavone) (19), luteolin 7-methyl ether (20) and chrysoeriol (5,7,4'-trihydroxy-3'-methoxyflavone) (21).

Considering the statement that a 5-hydroxyl group is essential for oestrogenic activity in isoflavones and the established fact that the flavone butrin has a clear antifertility activity it is reasonable to state that all the six flavones isolated from *Striga asiatica* may have oestrogenic activity.

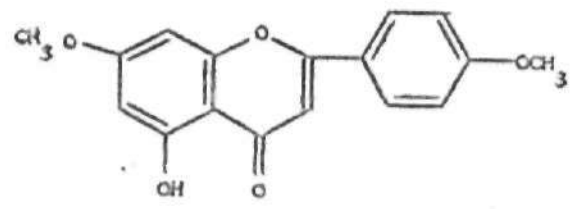
Among the plants recently being studied in Ayurveda and allopathy for their ability to prevent conception when taken orally is the plant *Striga lutea*, another species belonging to the family *Scrophulariaceae*. It has been observed that this plant material prevented conception when administered for three days during menstruation (Hiremath and Rao, 1990 b). Two flavones, acacetin (17) and luteolin (19) were isolated from the chloroform extract of this plant and were found to have significant antifertility activity in the pre-implantation stages of gestation in rats (Hiremath and Rao, 1990 b). This was the first time when a species of the family *Scrophulariaceae* was subjected to biological screening (Hiremath



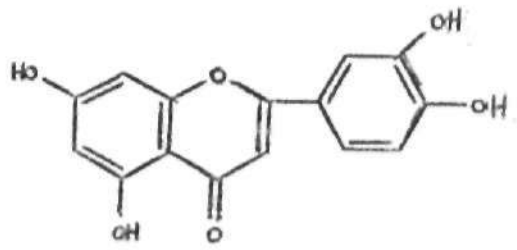
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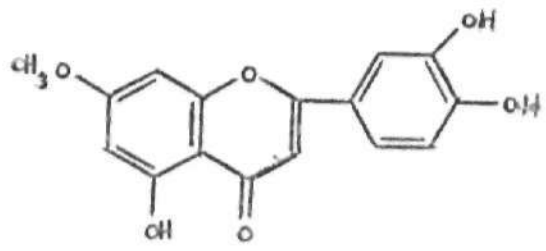
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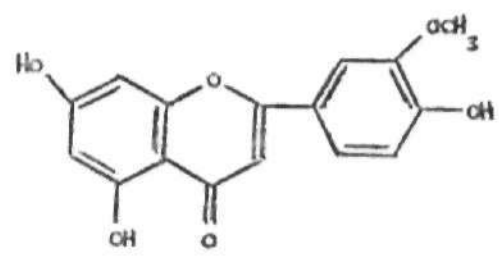
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(21)

and Rao, 1990 a). The anti-implantation activities of these two flavones may be due to their oestrogenic activity since, and it is interesting to note that, the compounds possessed 40-50 per cent oestrogenic potency as compared to oestradiol benzoate (Hiremath and Rao, 1990 b), which is a standard contraceptive drug.

1.2 Taxonomy / Medicinal Uses

Striga senegalensis is a species of the family *Scrophulariaceae* which is a large family of plants having 52 genera and some 2,500 species most of which are herbs or shrubs found throughout the world. Some members of this family of plants are annuals, some biennials and some are perennials. Among the genera of this family include *Alectra, Cycnium, Sopubia, Verbascum, Veronica* and *Striga*. The flowers of the members of this family are zygomorphic, or bilaterally symmetrical, with the calyx and corolla both tubular and each composed of four or five lobes. Many of the species of the family are parasites or semi-parasites which draw nourishment from the roots of grasses and, perhaps, other plants such as cereals. Plants of this family often turn black when dried. Drugs of medicinal value are found in several species of the family, the most important, so far, being digitalis, from the species of foxglove. Others are poisonous herbs.



Plate 1: Close view of *Striga senegalensis* in Guinea-corn field



Plate 2: Full view of *Striga senegalensis* and showing the whole height of Guinea-corn



Plate 3: *Striga senegalensis* just *some* few minutes after up-rooting

The genus *Striga* has many species which include: *Striga asiatica* with bright red flowers (Collins, 1987); *S. lutea* with flowers usually bright yellow, occasionally red or white in well grown specimens (Theodore, 1967); *S. latericea* with large pink to salmon flowers; *S. baumannii*, flowers usually brownish-red with scale-like leaves; *S. linearifolia* with white to pale mauve flowers; *S. gesnerioides* with flowers mauve to pale pink, usually unbranched and without chlorophyl; and *S. senegalensis* having pale mauve to pinkish flowers (see Plates 1-3).

Striga senegalensis is an annual species and grows widely in the Northern parts of Nigeria usually in beans and guinea-corn fields, where it is seriously parasitic and badly damaging rhe cereal. The infusion of the whole plant is used in Nigeria for curing stomach-ache in animals especially goats and sheep. In some parts of the country the plant material is also used about 3 months after pregnancy to induce abortion or mis-carriage of birth. To our knowledge no phytochemical or pharmacological studies have been carried out and reported on this plant and hence it has become our interest to subject the plant to such studies.

CHAPTER TWO

EXPERIMENTAL

2.1 Extraction/Chemical Studies of Purified Ethanolic Extract (E)

The flowers of the plant *Striga senegalensis* were collected between October and November from guinea corn fields in some parts of Kano Municipal area, Nigeria. The flowers were dried in air under shade and used without powdering. All the organic solvents were purified by distillation prior to use.

Extraction

The air-dried flowers (100gm) were successively extracted with petroleum ether (60-80°C), benzene, chloroform, ethyl acetate and 95% ethanol using soxhlet apparatus in five batches of 20gm each for an average of 2.5 hours till the colour disappeared. Each batch was dried in air before extracting with another solvent. The crude extracts were labelled as PE, BZ, CL, EA, and ET, respectively.

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Purification of Crude Ethanolic Extract (ET)

ET (2gm) was shaken with chloroform and distilled water (1:1; 50ml) and the two layers were separated using a separatory funnel. The organic layer was evaporated in air to give a greyish solid (1.35gm). The aqueous layer was discarded. The solid mass was subsequently washed with petroleum ether and benzene. The solid left was dissolved in ethyl acetate and the solvent was evaporated to yield a brown coloured solid (0.88gm). This ethyl acetate extract was labelled as E which was used for phytochemical and pharmacological study on isolated guinea-pig ileum.

The crude ethanolic extract (ET) was used for antifertility studies on female albino rats.

2.2 Pharmacological Studies

Two biological studies were carried out: (1) In vitro study on isolated guinea-pig iluem, (2) In vivo antifertility study on female albino rats.

2.2.1 *In vitro studies on isolated guinea-pig ileum*

The aims and objectives of this work was to investigate whether any of the crude extracts of the flowers of *Striga senegalensis* possessed any pharmacological activity, like contraction or relaxation, on isolated guinea-pig ileum.

Animals

A single guinea-pig (male) weighed 302gm was locally purchased and starved for 24 hours before use.

Apparatus

Recording microdynamometer and transducer, tissue bath, perspex-chamber, dissecting knife and insulin syringes were used for the experiment.

Preparation of Extract solution

The crude ethanolic extract ET and E were dissolved in distilled water. Distilled water was added to PE, BZ and CL extracts (in which they were insoluble) followed by the addition of a few drops of Tween 80 (polysorbate 80) to obtain a suspension of each extract. Tween 80 was added to the aqueous solutions of ET and E to maintain a common vehicle for all the extracts. The stock solution were prepared as follows:

PE	15mg/2ml	ET	150mg/2ml
BZ	50mg/2ml	E	130mg/3ml
CL	75mg/2ml		

Methodology

The isolated guinea-pig ileum (about 3cm long) was quickly placed in a petri-dish containing the physiological salt solution (Tyrode solution). The tissue was then set up in the organ bath, and allowed to stabilize for 30 minutes. Graded doses of acetylcholine were added into the bath and the responses were recorded by the microdynamometer. The dose which produced maximum response (contraction) was noted. After washing-out the tissue bath with fresh physiological salt solution, graded doses of the test substance were then added into the organ bath and any response produced recorded. The tissue bath was again washed-out and doses of acetylcholine repeated and again after washing out the bath, doses of the next substance were added and responses again recorded. All the crude extracts (PE, BZ, CL, ET) and the purified crude ethanolic extract, E, were screened in this way.

2.2.2. *In vivo (Antifertility) studies of the Crude Ethanolic Extract (ET) on Female Albino Rats*

The main aims and objectives of this study was to find out the anti-implantation effect, if any, of the crude ethanolic extract, ET, when orally administered, in different doses, to pregnant female albino rats.

Makrials

i) Male and female albino rats.

ii) Olympus Tokyo Microscope with Oil-Immersion magnification (x100).

iii) Philip Harris Magnifying lens (B 4102019, focal length 250 mm and magnification x 2)

iv) Microscope glass slides, intragastric catheter, insulin syringes, hand latex surgical gloves.

v) Gum acacia, Giemsa's stain and copper wire loop

Methodology

The vaginal smears of the female albino rats (weighing 160-198 gm) were studied microscopically using Giemsa's stain for the oestrous cycle. The rats found in the oestrous phase of the cycle were caged with males in the ratio of 2:1. The females were examined the following morning for evidence of copulation. The animals which showed thick clumps of spermatozoa in vaginal smears were separated for the experiment. The day on which spermatozoa were detected in the vaginal smear was counted as day 1 of pregnancy. Fifteen (15) pregnant female albino rats were allocated to three groups of 5 each and treated as follows:

Group A - Vehicle control (received 1% gum acacia only)

Group B - 40mg/kg body weight of ET extract in 1% gum acacia

Group C - 80mg/kg body weight of ET extract in 1% gum acacia

A suspension of the plant extract in 1% gum acacia was prepared and each member of the groups received the above stated dose each day orally by means of intragastric catheter from day 1 to day 5 of pregnancy. The control group received 1.5ml of 1% gum acacia only each day from day 1 to day 5 of pregnancy. The animals in all the three groups were

laparotomized under ether anaesthesia on day 10 of pregnancy. Both horns of the uterus of each animal were observed for the number and sizes of implantation sites.

1% gum acacia was prepared by dissolving 1gm of the substance in 100ml of distilled water on warming.

CHAPTER THREE

RESULTS AND DISCUSSIONS

3.1 Extraction/Chemical Studies

Extraction

The results of the extraction carried out on the dried flowers (100gm) of the plant *Striga senegalensis* using different organic solvents are provided in Table 3.1.

Table 3.1: Colour and weight of the crude extracts

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Crude Extract	Weight (gm)	Physical appearance
Petroleum ether, PE	1.57	Pinkish cream (semi solid)
Benzene, BZ	0.68	Reddish-brown (solid)
Chloroform, CL	0.79	Dark green (solid)
Ethyl acetate, EA	1.54	Dark brown (solid)
Ethanol, ET	2.86	Dark brown (solid)
Total weight :	7.44gm	

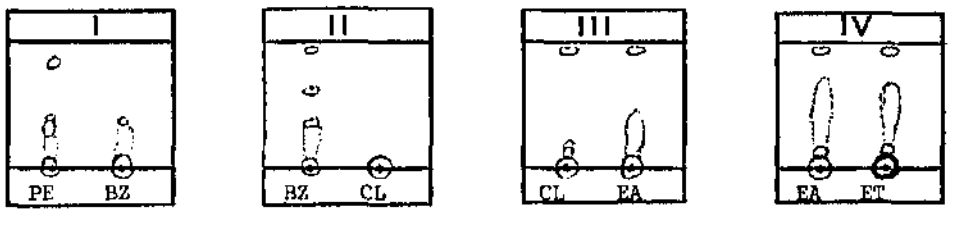
Thin Layer Chromatography (TLC)

The TLC pictures on silica gel pre-coated plastic plates (0.25mm, Camlab, West Germany) of the crude extracts are shown in Figure 3.1. The chromatograms were put in iodine chamber to develop the spots.

Purification of the Crude Ethanolic Extract, ET

Figure 3.2 shows the TLC picture of the chloroform layer (CL) using ethyl acetate (100%) as the solvent system. The TLC pictures of the extract, E

Figure 3.1: TLC for the crude extracts



Solvent systems

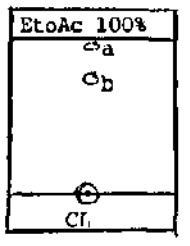
I Hexane: Ethyl acetate = 1:1

II Hexane: Ethyl acetate = 1:2

III Hexane: Ethyl acetate = 1:3

IV Hexane: Ethyl acetate = 1:4

Figure 3.2: TLC for the chloroform layer (CL)

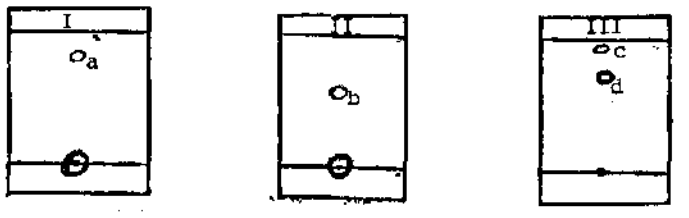


Solvent system: Ethyl acetate (100%)

Rf_a Value = 0.95

Rf_b Value = 0.80

Figure 3.3: TLC for the purified extract E



Solvent systems: I Ethyl acetate (100%)

II Benzene: Ethyl acetate = 1:1

III Ethyl acetate: Methanol = 1:1

Rf_a Value = 0.87

Rf_b Value = 0.56

Rf_c Value = 0.95

Rf_d Value = 0.75

(obtained from the purification of the chloroform layer), using three different solvent systems are provided in Figure 3.3.

Ultraviolet (UV) Spectroscopy

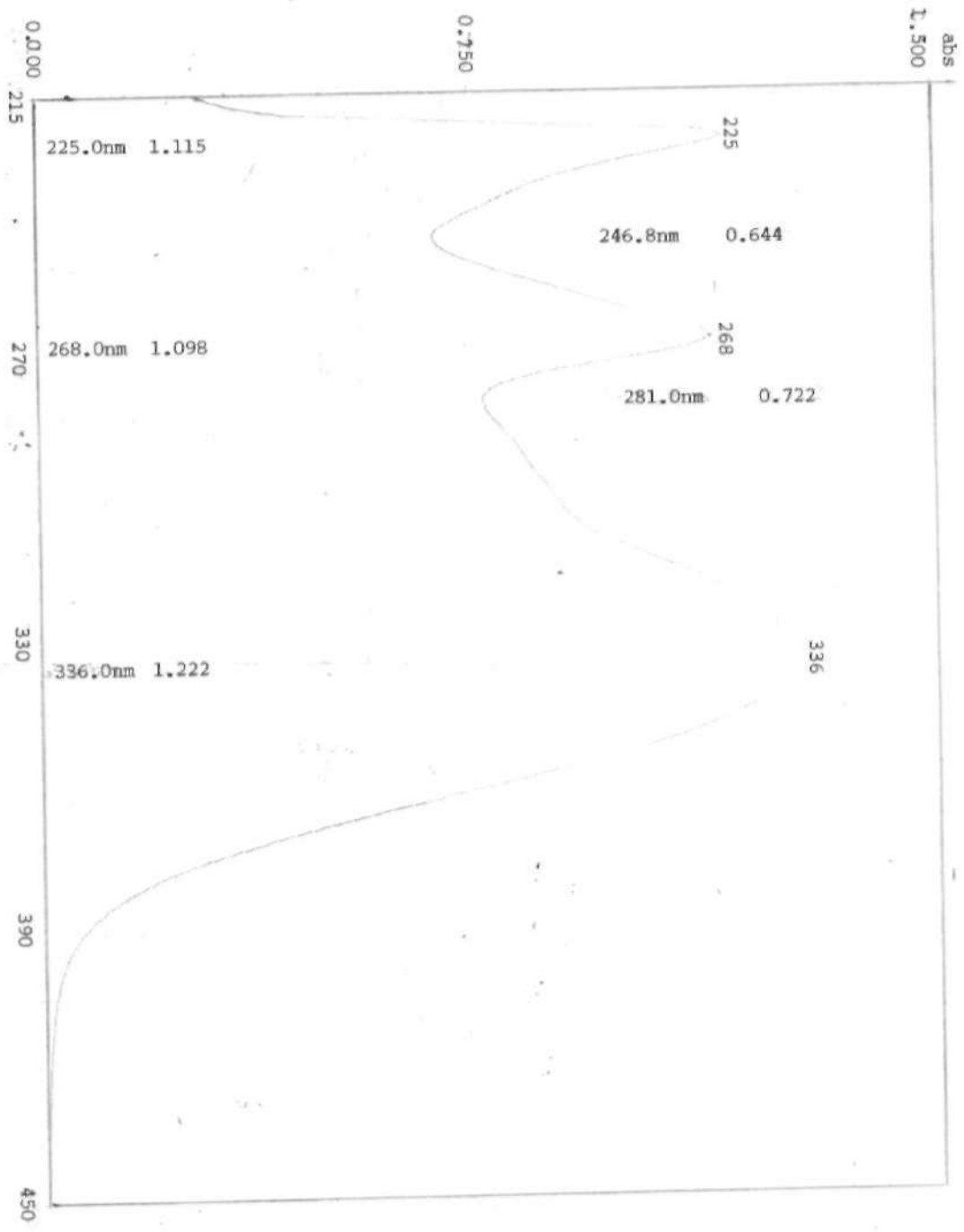
The UV absorption spectrum of E (Figure 3.4) was recorded in methanol using KONTRON UVIKON 860 Spectrophotometer. The absorption maxima , λ_{max} 225(1.115), 268 (1.098) and 336(1.222) nm observed in the spectrum indicated the presence of flavone(s), very consistent with a 5,7-dihydroxyflavone structure, and eliminated the presence of isoflavone (Scott, 1967). The flavones, apigenin (16) and acacetin (17) are both 5,7-dihydroxyflavones and both have λ_{max} (in methanol) of 268nm and 335nm. Another flavone, acacetin 7-methyl ether (18) has been found to have λ_{max} values of 269 and 335nm (Dhar et al., 1970). Flavones show two UV absorption bands: Band I, 330-350nm and Band II, 250-270nm (Finar, 1986).

Colour test

Colour reactions are not usually strictly characteristics for flavones since a given class of flavonoids will produce colours according to the hydroxylation pattern and also the presence of other substituents. However, the Shinoda Test has been useful in distinguishing individual flavones in a given group. Using this test flavonoid compounds containing hydroxyl or methoxyl substitution in the 2-phenyl group usually give red to violet colours while a 5,7-dimethoxyflavone produces a pink colour (Shinoda, 1928).

Sample E

Figure 3.4: UV Absorption Spectrum of E



Sample E

(1) *Ferric Chloride (10%) Test*

Ethanolic solution of E gave violet colour wih ferric chloride solution indicating the presence of phenolic hydroxyl group(s).

(2) *Sodium hydroxide and Concentrated Sulphuric acid test*

Ethanolic solution of E produced yellow colour on addition of sodium hydroxide (2M) solution to it. The ethanolic solution of E gave orange solution with concentrated sulphuric acid indicating the presence of phenolic -OH group (s).

(3) *Shinoda Test*

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A few drops of hydrochloric acid when added to the ethanolic solution of E, containing a few magnisium chips, in a test tube produced a pale red solution. This pale red colour did not fade for some time but was changed to violet colour on addition of a few drops of sodium acetate solution. The violet colour changed to yellow on addition of a few drops of NaOH (2M) and the yellow colour was decolourized by the addition of a few drops of hydrochloric acid solution.

It can be inferred from the above results and the values of the λ_{max} in the UV spectrum obtained that E could contain 5,7-dihydroxy flavonoid compound(s) having either a hydroxy or methoxy function in the 2-phenyl group.

Infra-red (IR) Spectroscopy

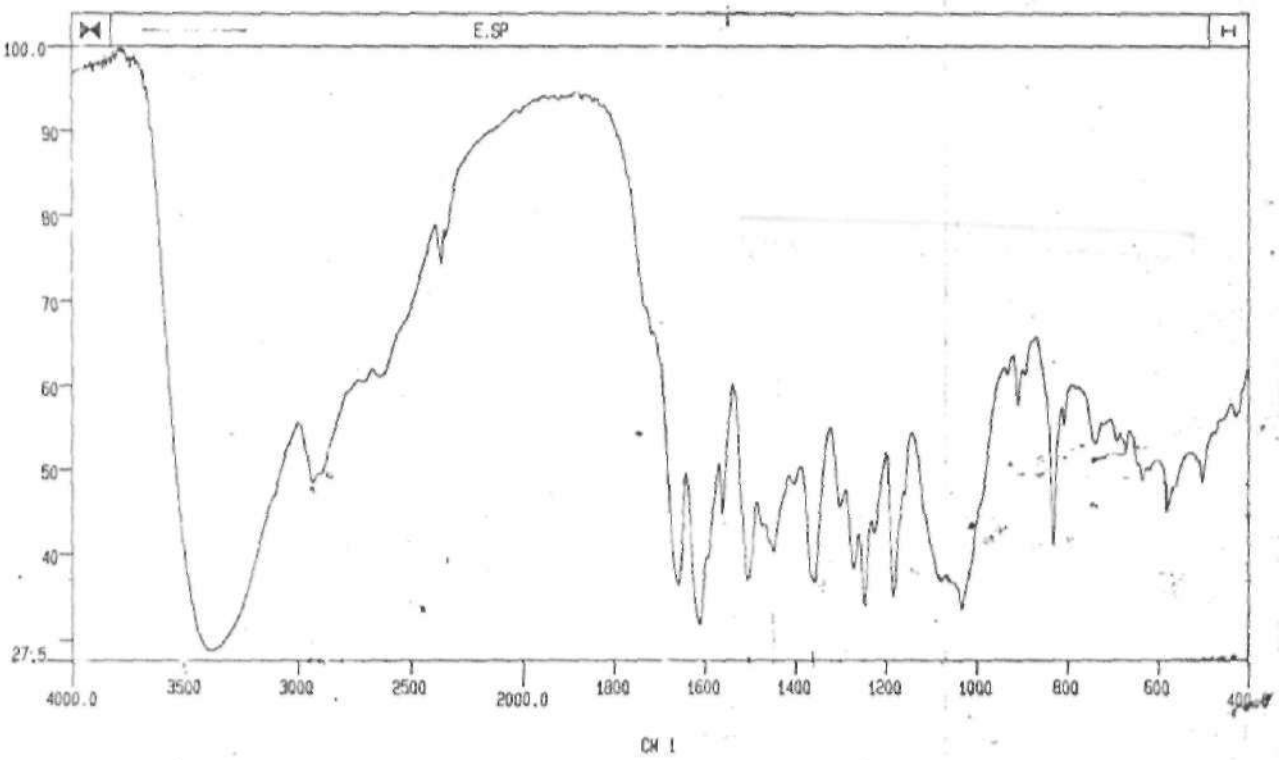
The IR spectrum of E (Figure 3.5) was recorded with KBr disc using a Perkin Elmer 1725 FT-IR Spectrometer model. The identified diagnostic absorption bands for flavones in the spectrum are provided in Table 3.2.

Table 3.2: Flavones diagnostic IR absorption bands in the spectrum of E

Absorption band (cm^{-1})	Inference
3381 cm^{-1}	O–H Stret. (Intermolecular H-bonding)
1656 "	C=O absorption (Conjugated)
1609 " 1558 " 1505 "	C=C Stret. (in-plane aromatic skeletal vibrations)
1245 "	C–O Stret. (aromatic)
1031 "	C–O Stret. (aliphatic)

Since the O–H absorption band does not overlap the C–H stretching absorption region and is sharp, lying in the region 3500-3200 cm^{-1} there must probably be fewer hydroxyl groups. The absorption bands at 1269cm and 1031 cm^{-1} suggested the presence of methoxy (–OCH) substitution. These diagnostic IR absorption peaks of E correlate with those of many flavones. For instance, the following IR absorption bands have been noted for the flavone apigenin (16) : 1660cm^{-1} (C=O, conjugated), 1250cm^{-1} (OCH) and 1600cm (C=C, aromatic) (Scheinmann, 1973). The flavone, acacetin 7-methyl ether (18) has IR absorption bands 1687cm^{-1} (C=O, conjugated), 1605cm^{-1} (C=C, aromatic), and 1250cm^{-1} and 1408cm^{-1} (OCH).

Figure 3.5: IR Absorption Spectrum of E



E.SP 3501 4000.00 100.00 28.69 99.91 4.00 %T 16 2.000

REF 4000 96.87 2000 92.88
3381.00 28.69 2926.00 48.37 2361.00 74.51 1656.00 36.40 1609.00 31.82
1558.00 44.77 1505.00 36.93 1446.00 40.28 1356.00 36.74 1299.00 45.52
1269.00 38.33 1245.00 34.09 1224.00 42.46 1182.00 35.22 1031.00 33.68
908.00 57.52 829.00 41.00 806.00 55.23 737.00 53.11 670.00 52.02
633.00 48.42 578.00 45.09 501.00 48.40 427.00 56.31
END 24 PEAK(S) FOUND

GC - MASS Spectrometry

The TLC pictures of E (Figure 3.3) have indicated two distinct spots with the most dense spot at the base (Plates I and II) and the lighter spot having an Rf value of 0.87 (Plate I) and 0.56 (Plate II). GC - Mass spectrometer was used to separate component(s) of these two spots.

A SATURN 3 GC/MS equipped with split injector and flame ionization detector (FID) was used. The column used was liquid phase DB -1 with film thickness of 1.00 µm and dimension of 30m x 0.251mm having temperature limits of - 60°C to 325°C. However, the column temperature used for this work was between 80°C to 250°C with an increase rate of 3°C per minute. The temperature of the injector and detector were set at 250°C and 260°C, respectively. Helium was used as a carrier gas at a flowrate of 1.1ml/ minute or 37.5cm/second.

The GC Chromatogram plotted between 1 to 1499 scan numbers for E is provided in Figure 3.6. When this chromatogram was blown no additional peaks were recorded in the range 100 to 600 scan numbers but peaks now appeared in the region 600 to 1499 scan numbers. The blown chromatograms are provided in figure 3.7 (blown 100 to 600 scan numbers range) and Figure 3.8 (blown 600 to 1499 scan numbers range).

The mass spectra corresponding to some of the peaks in the blown chromatograms of E are given in Figures 3.9, 3.10, and 3.11 in the order of increasing scan numbers. Computer search has correlated these mass spectra of the components of E with those of some known compounds and this correlation is summarized and provided in Table 3.3.

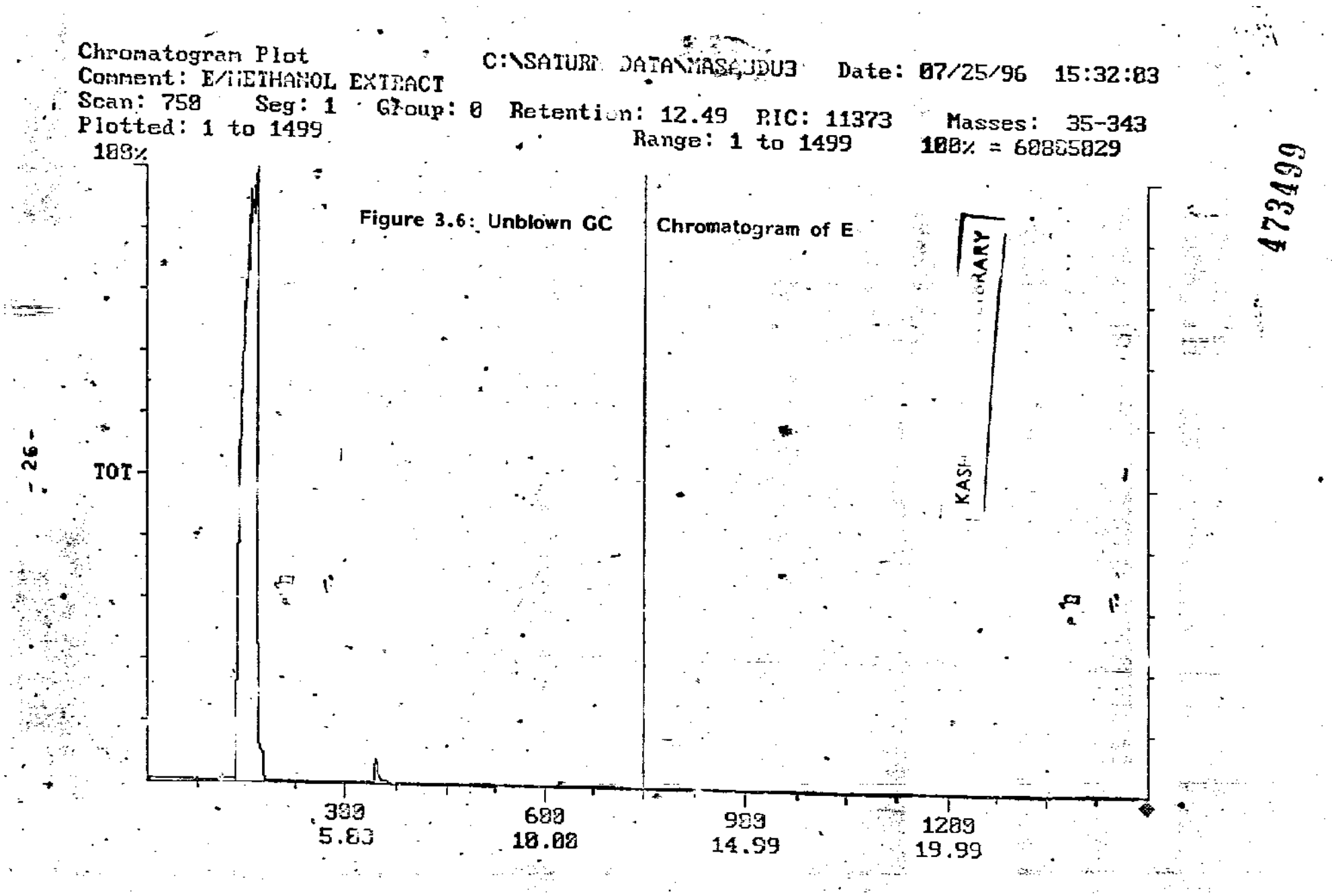


Figure 3.6: Unblown GC Chromatogram of E

473499

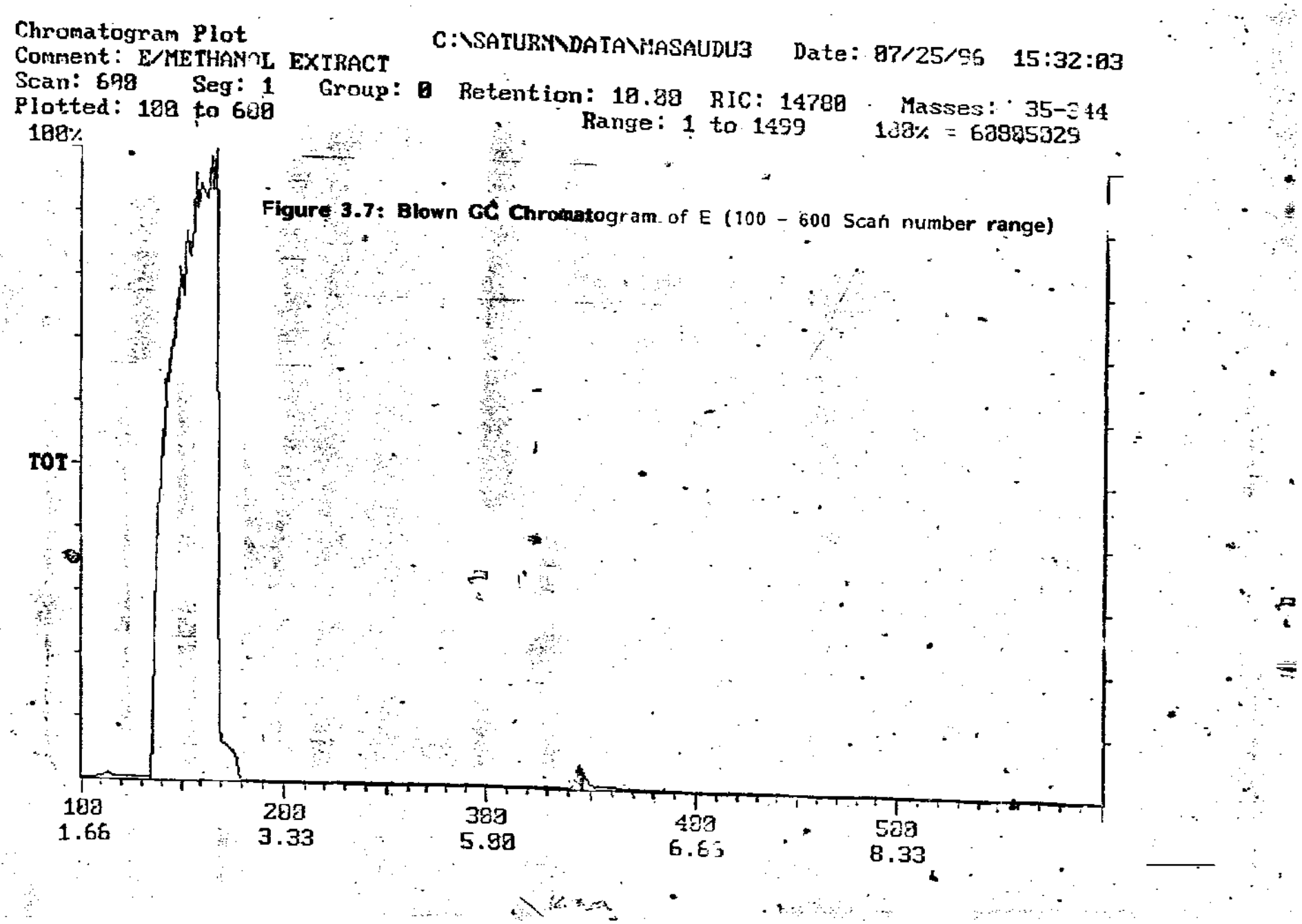


Figure 3.7: Blown GC Chromatogram of E (100 – 600 Scan number range)

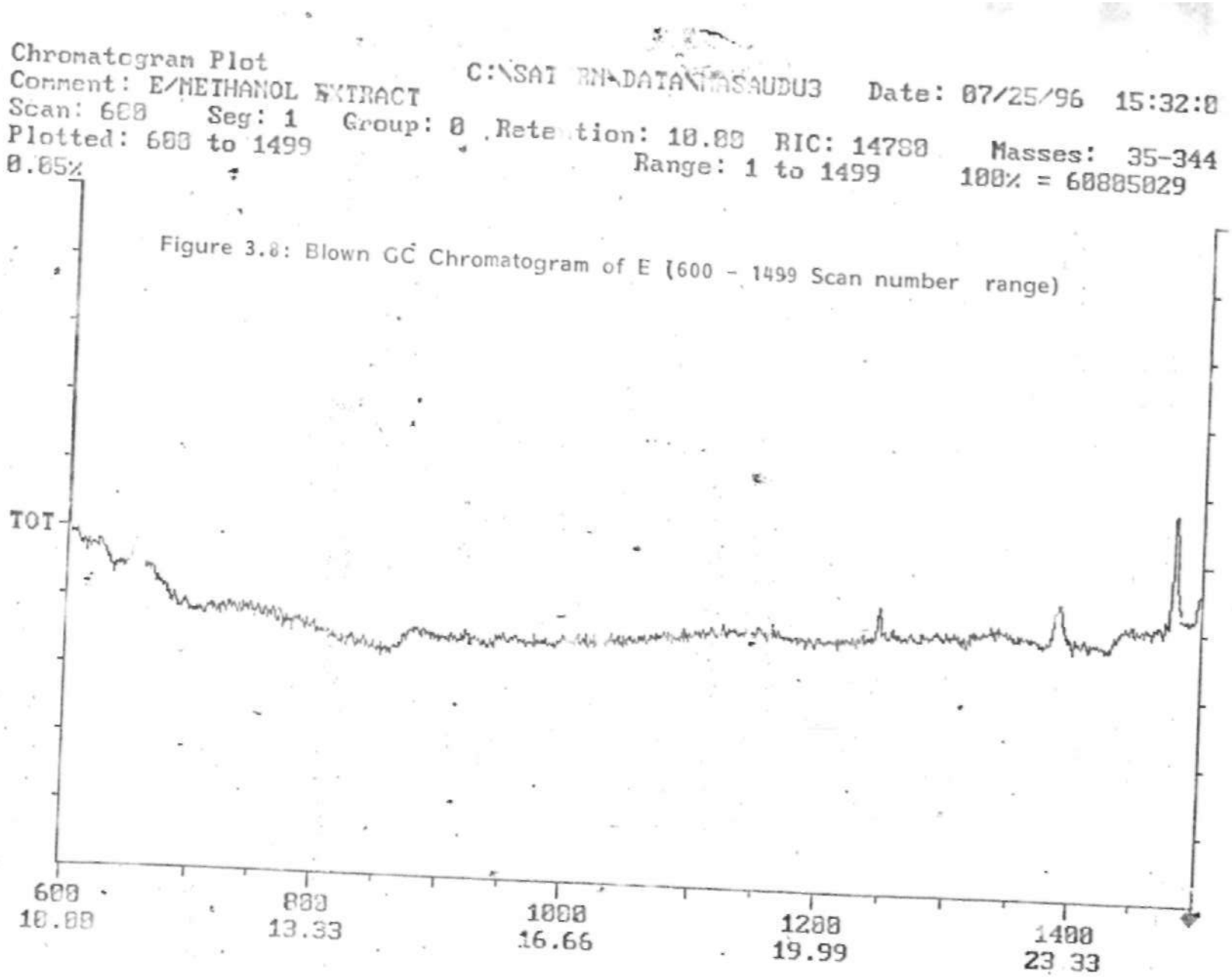


Figure 3.8: Blown GC Chromatogram of E (600 - 1499 Scan number range)

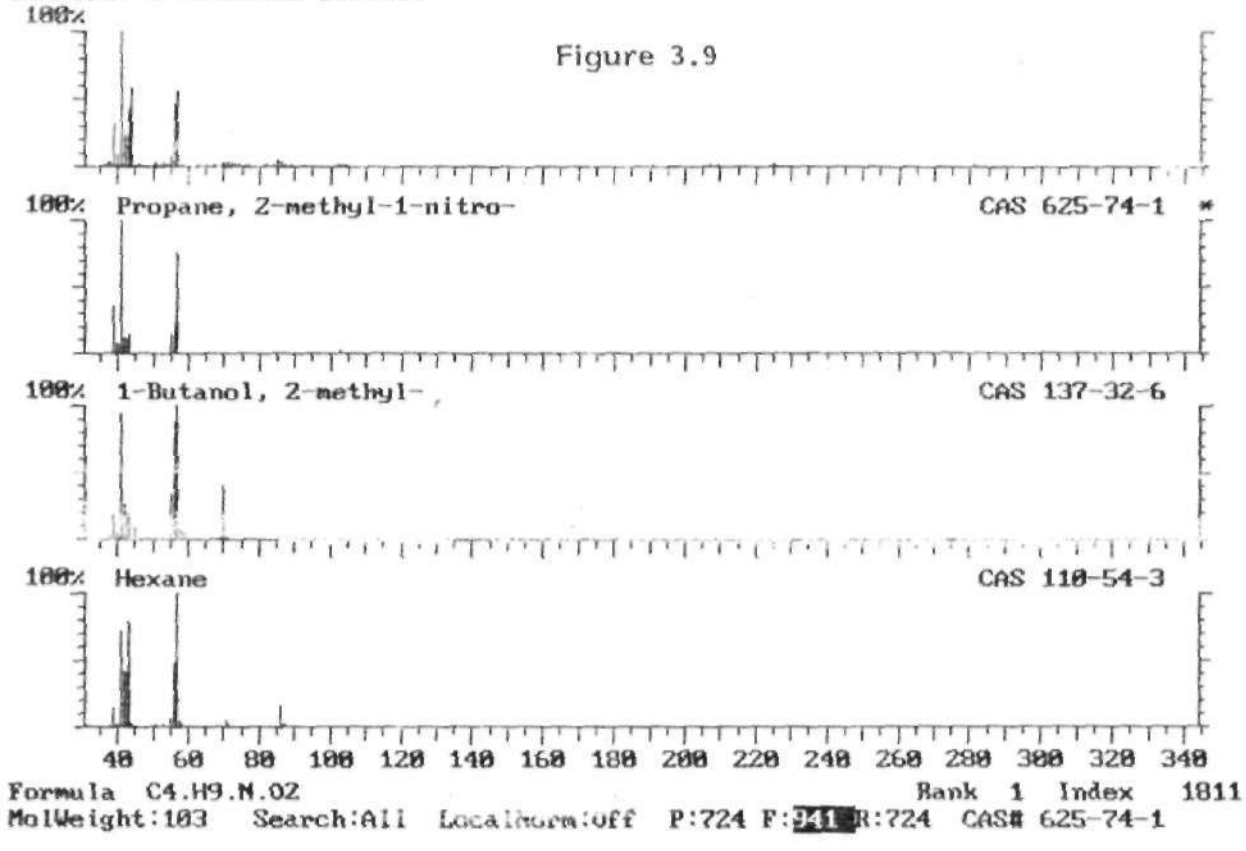


Figure 3.9

Search Results Datafile:MASAUDU3 Spectrum:327 Libr:NIST92 Range:All

(1) Propane, 2-methyl-1-nitro-
1811 Purity: 724 Fit: 941 Rfit: 724 CAS# 625-74-1
Formula: C4.H9.N.O2 Mol Wt: 103

(2) 1-Butanol, 2-methyl-
40682 Purity: 668 Fit: 831 Rfit: 678 CAS# 137-32-6
Formula: C5.H12.O Mol Wt: 88

(3) Hexane
40687 Purity: 786 Fit: 814 Rfit: 787 CAS# 110-54-3
Formula: C6.H14 Mol Wt: 86

(4) Hydroxylamine, O-(2-methylpropyl)-
879 Purity: 757 Fit: 798 Rfit: 789 CAS# 5618-62-2
Formula: C4.H11.N.O Mol Wt: 89

(5) Butane, 2,2-dimethyl-
40595 Purity: 726 Fit: 761 Rfit: 729 CAS# 75-83-2
Formula: C6.H14 Mol Wt: 86

(6) Pentane, 2,2,3,4-tetramethyl-
42879 Purity: 696 Fit: 724 Rfit: 728 CAS# 1186-53-4
Formula: C9.H20 Mol Wt: 128

(7) Oxirane, [(2-propenyloxy)methyl]-
41855 Purity: 658 Fit: 707 Rfit: 673 CAS# 106-92-3
Formula: C6.H10.O2 Mol Wt: 114

(8) Pentane, 2,2,3,4-tetramethyl-
5163 Purity: 685 Fit: 706 Rfit: 731 CAS# 1186-53-4
Formula: C9.H20 Mol Wt: 128

(9) Oxirane, 2-methyl-3-propyl-, trans-
1532 Purity: 642 Fit: 700 Rfit: 675 CAS# 6124-91-0
Formula: C6.H12.O Mol Wt: 100

(10) Hexane, 3-methyl-
41155 Purity: 673 Fit: 694 Rfit: 687 CAS# 589-34-4
Formula: C7.H16 Mol Wt: 100

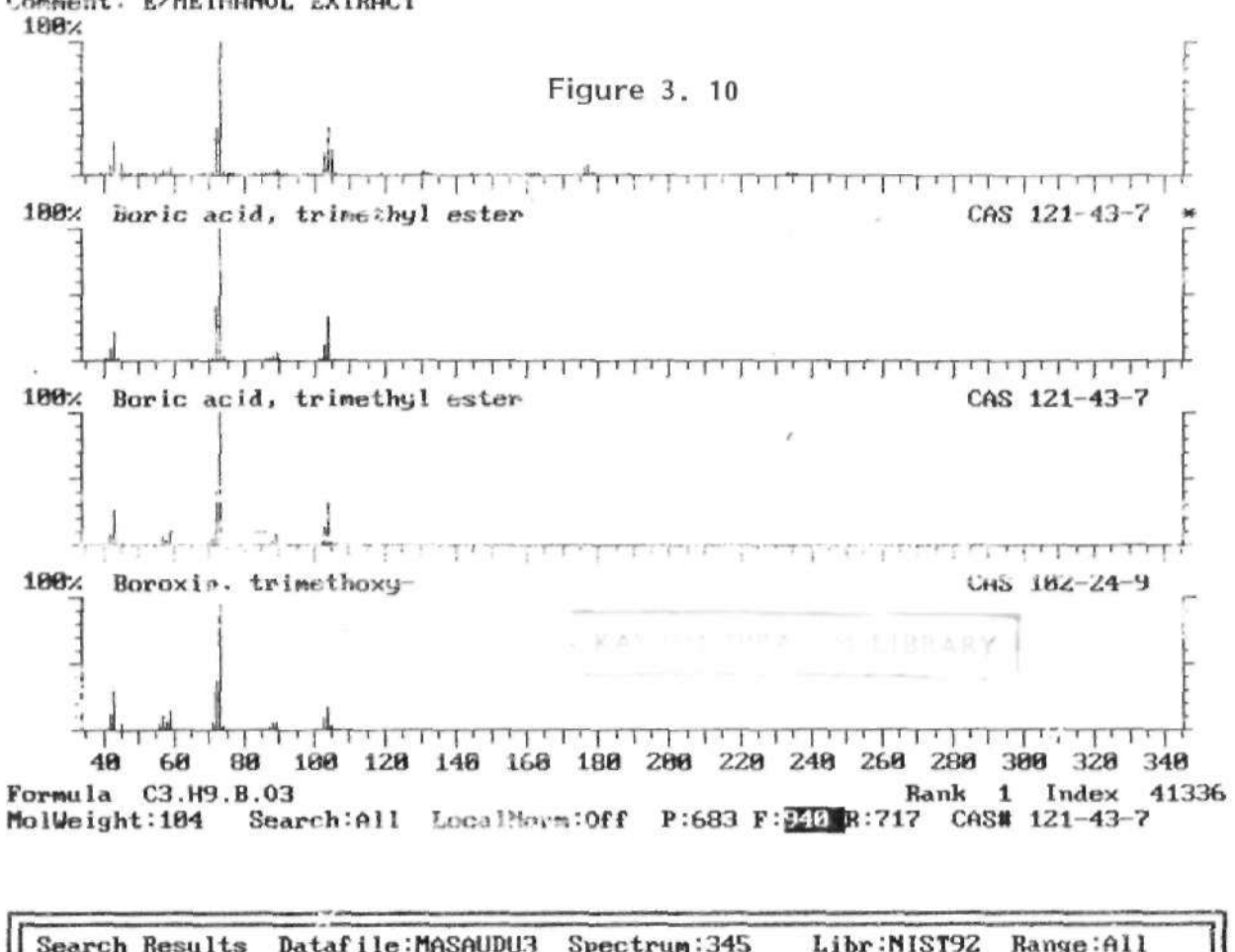


Figure 3. 10

Search Results Datafile:MASAUDU3 Spectrum:345 Libr:NIST92 Range:All

(1) Boric acid, trimethyl ester
41336 Purity: 683 Fit: 940 Rfit: 717 CAS# 121-43-7
Formula: C3.H9.B.03 Mol Wt: 104

(2) Boric acid, trimethyl ester
1853 Purity: 775 Fit: 917 Rfit: 737 CAS# 121-43-7
Formula: C3.H9.B.03 Mol Wt: 104

(3) Boroxin, trimethoxy-
16820 Purity: 755 Fit: 915 Rfit: 755 CAS# 102-24-9
Formula: C3.H9.B3.06 Mol Wt: 174

(4) Acetamidoacetaldehyde
1605 Purity: 394 Fit: 817 Rfit: 413 CAS# 64790-88-5
Formula: C4.H7.N.02 Mol Wt: 101

(5) Silane, diethylmethyl-
1758 Purity: 463 Fit: 773 Rfit: 488 CAS# 760-32-7
Formula: C5.H14.Si Mol Wt: 102

(6) Propane, 2,2-dimethoxy-
41359 Purity: 364 Fit: 732 Rfit: 415 CAS# 77-76-9
Formula: C5.H12.02 Mol Wt: 104

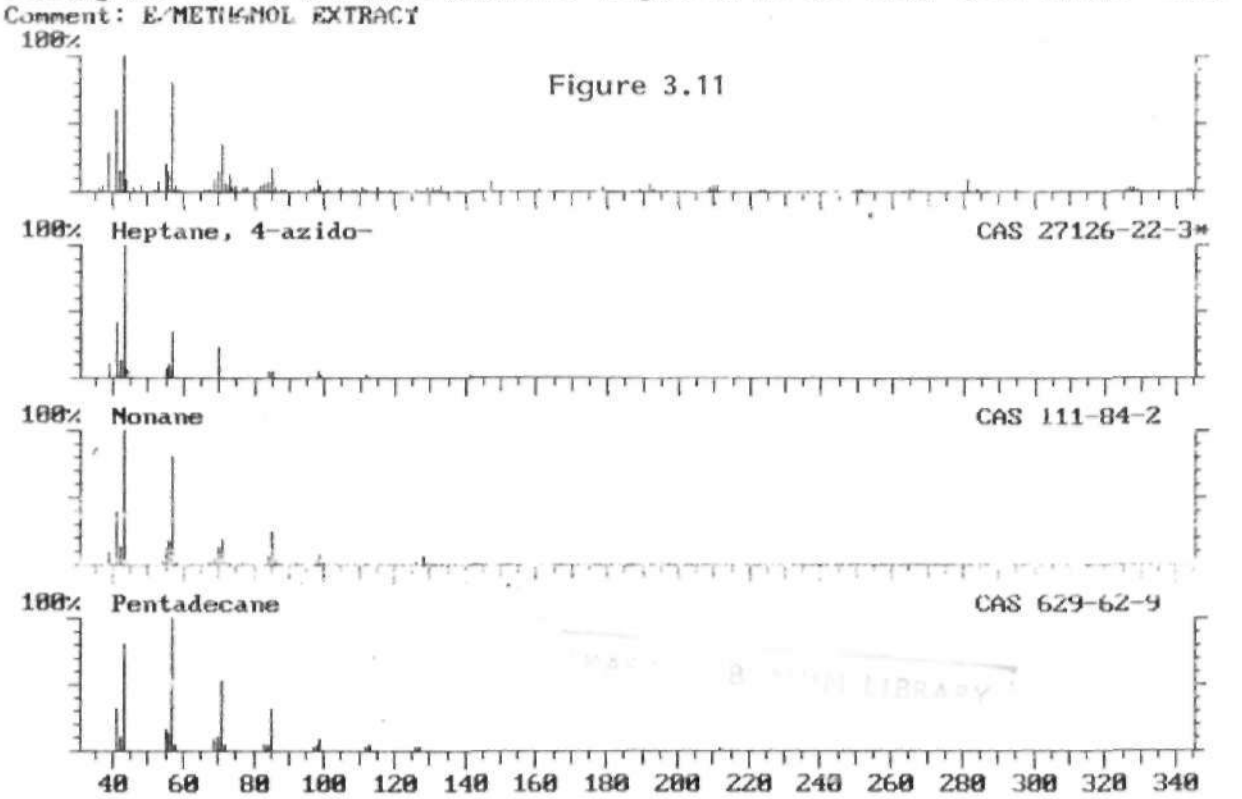
(7) Butanedioic acid, hydroxy-, dimethyl ester
67627 Purity: 126 Fit: 678 Rfit: 155 CAS# 1587-15-1
Formula: C6.H10.05 Mol Wt: 162

(8) Scyllo-Inositol
17572 Purity: 406 Fit: 65- Rfit: 499 CAS# 488-59-5
Formula: C6.H12.06 Mol Wt: 180

(9) Benzene, 1,1'-(1,2-cyclobutanediyl)bis-, cis-
25031 Purity: 275 Fit: 643 Rfit: 308 CAS# 7694-30-6
Formula: C16.H16 Mol Wt: 208

(10) (Ss)- or (rr)-4-methyl-2,3-pentanediol
3542 Purity: 314 Fit: 638 Rfit: 376 CAS# 6464-48-0
Formula: C6.H14.02 Mol Wt: 118

Figure 3.11



Search Results Datafile:MASAUDU3 Spectrum:1245 Libr:NIST92 Range:All

(1) Heptane, 4-azido-					
# 7692	Purity: 456	Fit: 934	Rfit: 458	CAS# 27126-22-3	
Formula: C7.H15.N3				Mol Wt: 141	
(2) Nonane					
# 42878	Purity: 546	Fit: 908	Rfit: 573	CAS# 111-84-2	
Formula: C9.H20				Mol Wt: 128	
(3) Pentadecane					
# 70273	Purity: 505	Fit: 902	Rfit: 535	CAS# 629-62-9	
Formula: C15.H32				Mol Wt: 212	
(4) Heptadecane, 2-methyl-					
# 71546	Purity: 503	Fit: 898	Rfit: 550	CAS# 1560-89-0	
Formula: C18.H38				Mol Wt: 254	
(5) Hydroxylamine, O-(2-methylpropyl)-					
# 879	Purity: 478	Fit: 888	Rfit: 506	CAS# 5618-62-2	
Formula: C4.H11.N.O				Mol Wt: 89	
(6) Butane, 2,2-dimethyl-					
# 40595	Purity: 466	Fit: 882	Rfit: 473	CAS# 75-83-2	
Formula: C6.H14				Mol Wt: 86	
(7) Tridecane					
# 69019	Purity: 491	Fit: 882	Rfit: 536	CAS# 629-50-5	
Formula: C13.H28				Mol Wt: 184	
(8) Undecane					
# 67317	Purity: 507	Fit: 878	Rfit: 555	CAS# 1120-21-4	
Formula: C11.H24				Mol Wt: 156	
(9) Hexadecane, 3-methyl-					
# 32856	Purity: 495	Fit: 877	Rfit: 546	CAS# 6418-43-5	
Formula: C17.H36				Mol Wt: 240	
(10) Decane, 2,5,6-trimethyl-					
# 19017	Purity: 548	Fit: 876	Rfit: 569	CAS# 62108-23-0	
Formula: C13.H28				Mol Wt: 184	

Table 3.3: Computer Implicated compounds in E

Scan Number	Implicated Comound	% Fitness	M wt
327	2-methyl-1-nitropropane	94.00	103
345	Boric acid, trimethyl ester (methyl borate or trimethyl borate)	94.00	104
1245	Nonane	90.80	128

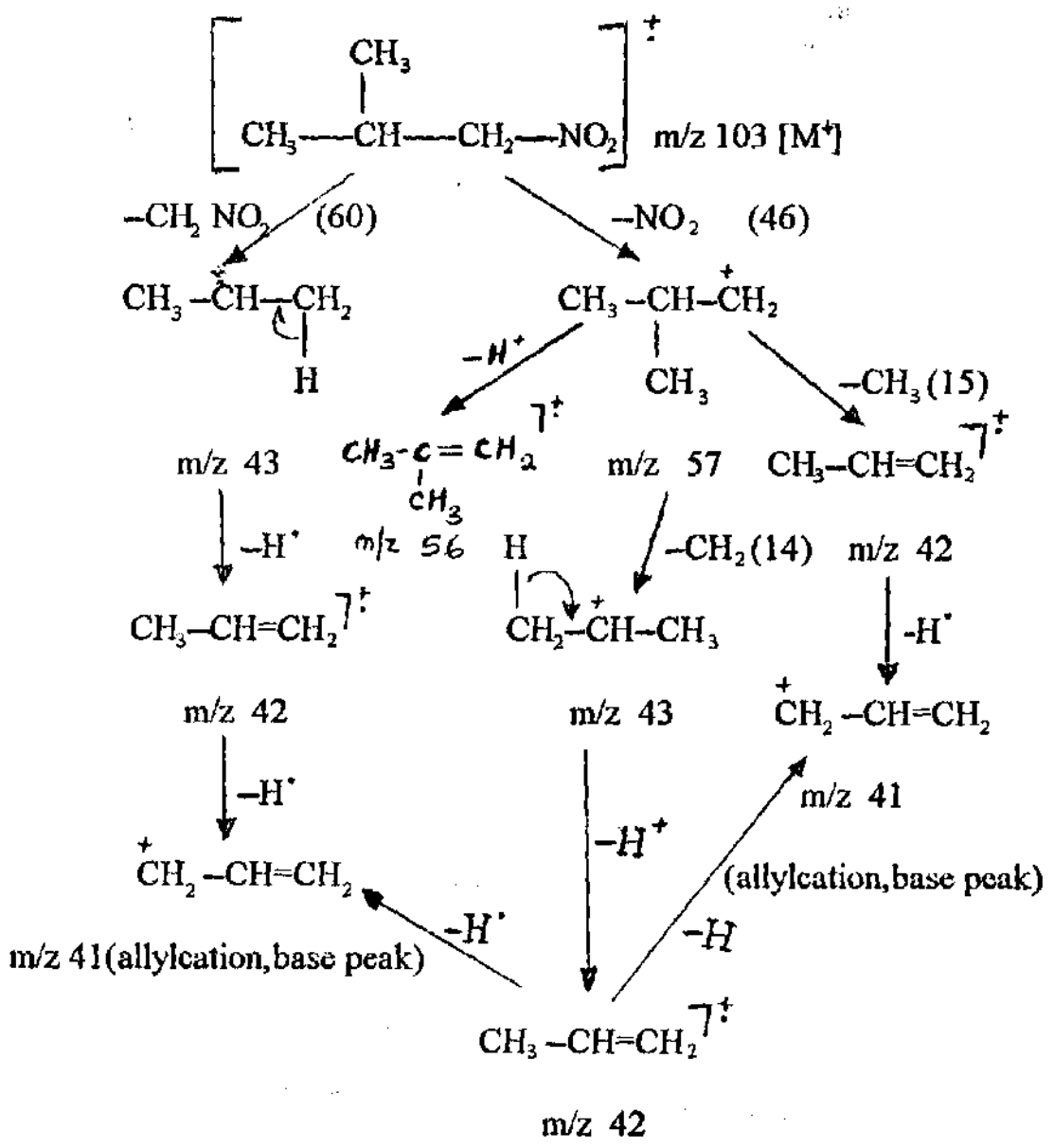
The mass fragmentation patterns to justify the mass peaks in the mass spectra of the compounds implicated to be 2-methyl-1-nitropropane, methyl borate (trimethyl borate or boric acid trimethyl ester) and nonane are given in schemes 3.1,3.2 and 3.3, respectively.

The mass spectrum of the component of E implicated to be n[illegible]e (with 90.8% fitness) has ion peaks at m/z 43 (100%, base peak), 39 (27.8), 41 (61), 57 (77.8), 71 (33.3), 85 (16.7) and 99 (5.6). Trimethyl borate (the implicated compound) has the fragment ions distribution worked-out as : m/z 73 (100%, base peak), 104 (36.4), 103 (18.2), 89 (5.5), 72 (36.4), 43 (25.5) and 42 (9.1). Similarly the fragment ions distribution can be measured-out from the mass spectrum of the component of E computer implicated to be 2-methyl-1-nitropropane (with 94% fitness) and be presented as: m/z 41 (100%, base peak), 55 (8.3), 57 (55.6), 56 (47.2), 44 (61.1), 43 (44.4), 42 (27.8), 39 (33.3), 56 (43), and 57 (58.3).

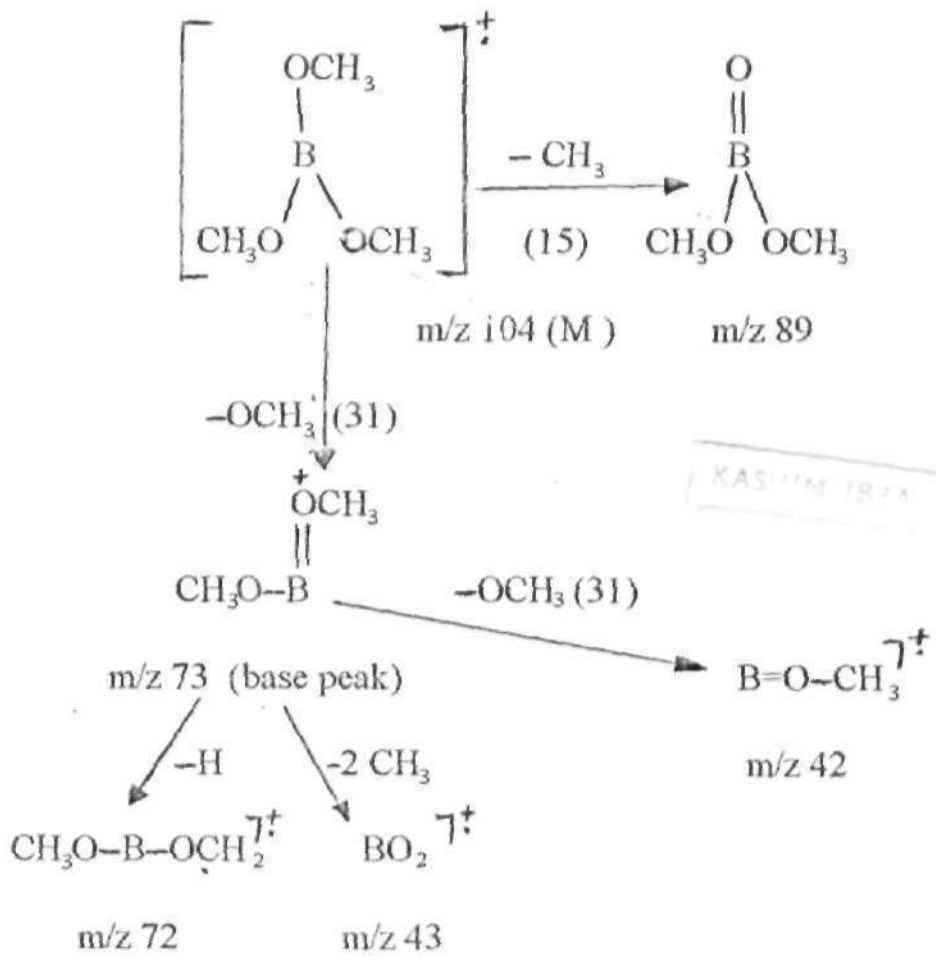
Boron is one of the micronutrients required by plants and despite the small amounts needed they are no less essential than the macronutrients because plants cannot survive without them. Insufficiency of boron will lead to total suppression of flowering while fruits and seeds formation, if it occurs, will

[illegible]

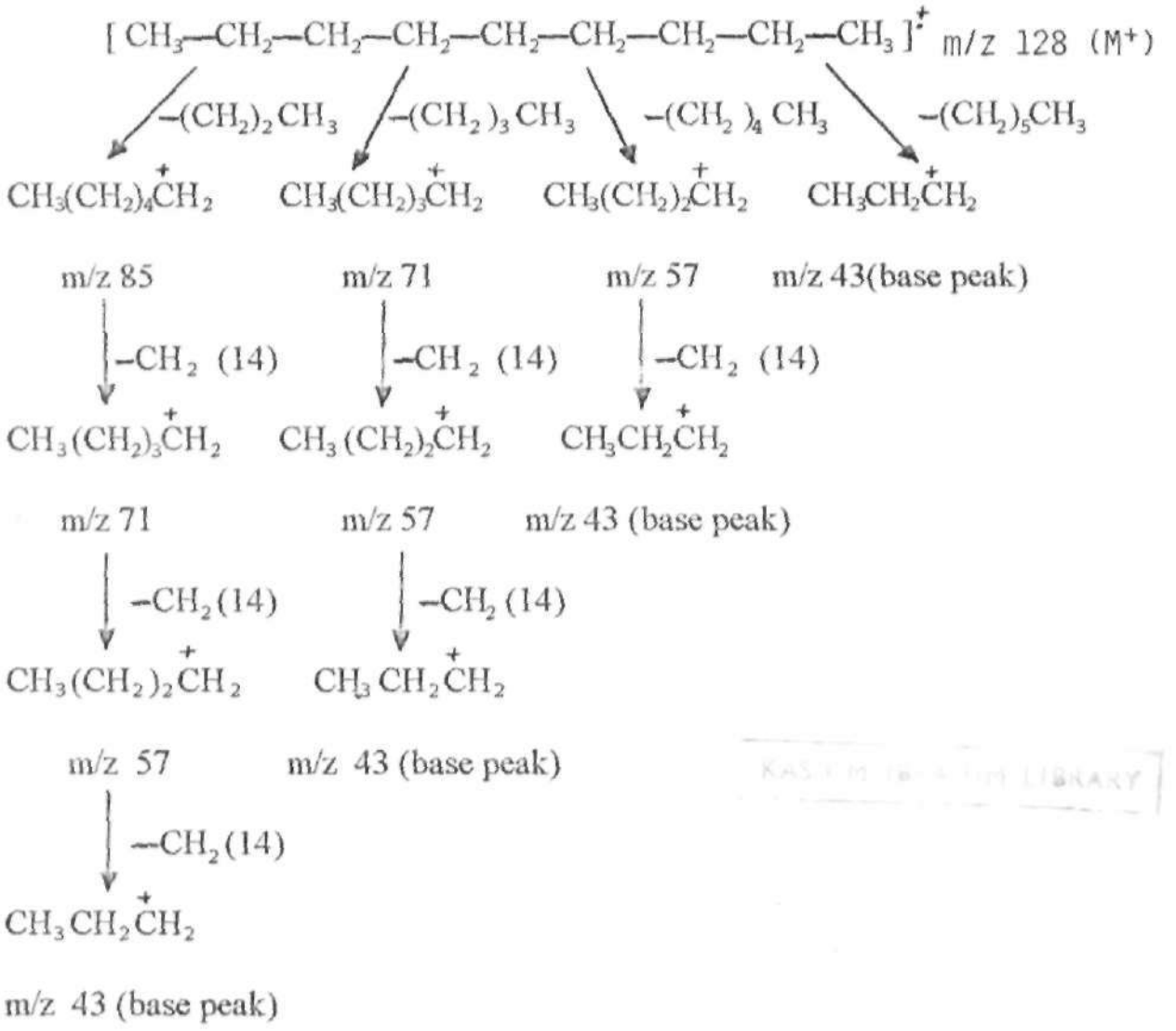
Scheme 3.1: Fragmentation Pattern for 2-methyl-1-nitropropane



Scheme 3.2: Fragmentation Pattern for Trimethyl borate



Scheme 3.3: Fragmentation Pattern for nonane



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normally be abnormal (Sutclffe and Baker, 1978).Gauch and Dugger (1953) put forward a theory that boron forms a complex with sugars that penetrates into plant living cells more readily than do free sugars and is therefore more readily translocated to the growing cells. However, most experimental evidences appear to oppose this theory and many other explanations on the functions of boron in plants have been proposed. According to Lee and Aronoff (1967), borate ion inhibits an enzyme system which in the absence of boron, leads to excess formation of phenolic acids and to necrosis and eventual death of the plant. It has also been proposed that boron is reguired for flavonoid synthesis (Rajaratnam et al., 1971).

Electron impact mass spectrometry of both flavonoid aglycones and glycosides serves as a valuable aid in determining their structures, especially when only very small quantities of the compounds are available. The method has been applied successfully to all classes of flavonoid aglycones (Porter and Baldas, 1971). Most flavonoid compounds yield intense peaks for the molecular ion and indeed this is often the base peak. Derivatization is thus unneccessary unless GC/Mass spectrometry is to be carried out in which case a suitable derivatization method to provide adequate volatility should be employed. This is one major requirement when using GC/Mass spectrometry for flavonoids. It is perhaps because this has not been taken care of that the analysis of Sample E did not provide mass spectrum of any flavonoid

despite the fact that all other tests carried out on E indicated the probable presence of flavonoid(s). Trimethylsilylation (Pellizzari et al., 1969) or permethylation (Schmid and Harbone, 1973) derivatization techniques have been successfully applied to flavonoids to provide the desired volatility.

3.2 Pharmacological Studies

In vitro Studies on Isolated Guinea-pig Ileum

The results of the in vitro pharmacological screening of the crude extracts (PE, BZ, CL and ET) and the purified ethanolic extract (E) are provided in figures 3.12 to 3.16. The results indicated that the extracts PE, BZ, and CL did not have any activity on the guinea-pig ileum even at the dose levels of 6mg, 1.25mg and 30mg, respectively. Comparable dose-dependent contraction activities with acetylcholine (Ach), Figure 3.17, have been shown by the extracts ET and E. The doses of Ach, ET and E and their corresponding responses on guinea-pig ileum are given in Table 3.4.

Figure 3.12: Activity of PE on Guinea-pig ileum

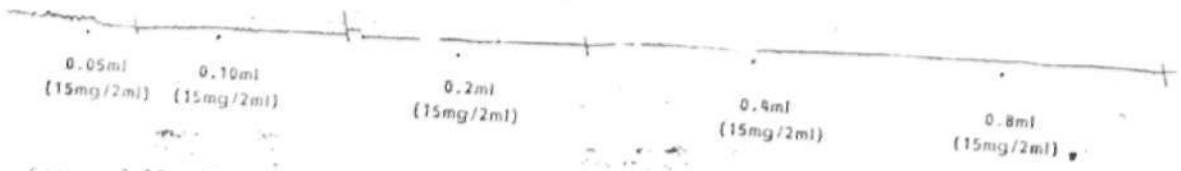


Figure 3.13: Activity of BZ on Guinea-pig ileum

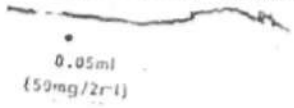


Figure 3.14: Activity of CL on Guinea-pig ileum

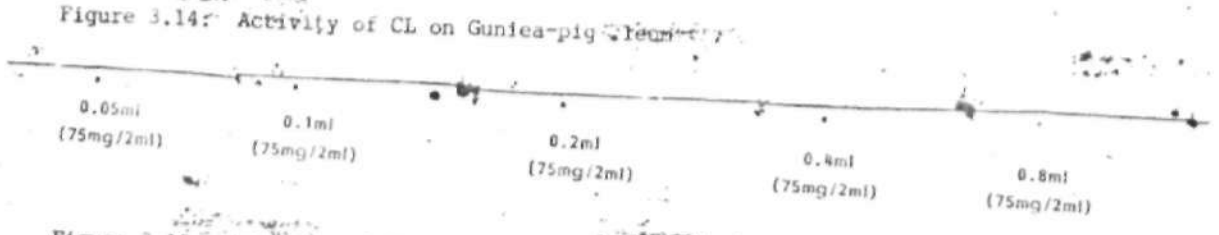


Figure 3.15: Activity of ET on Guinea-pig ileum

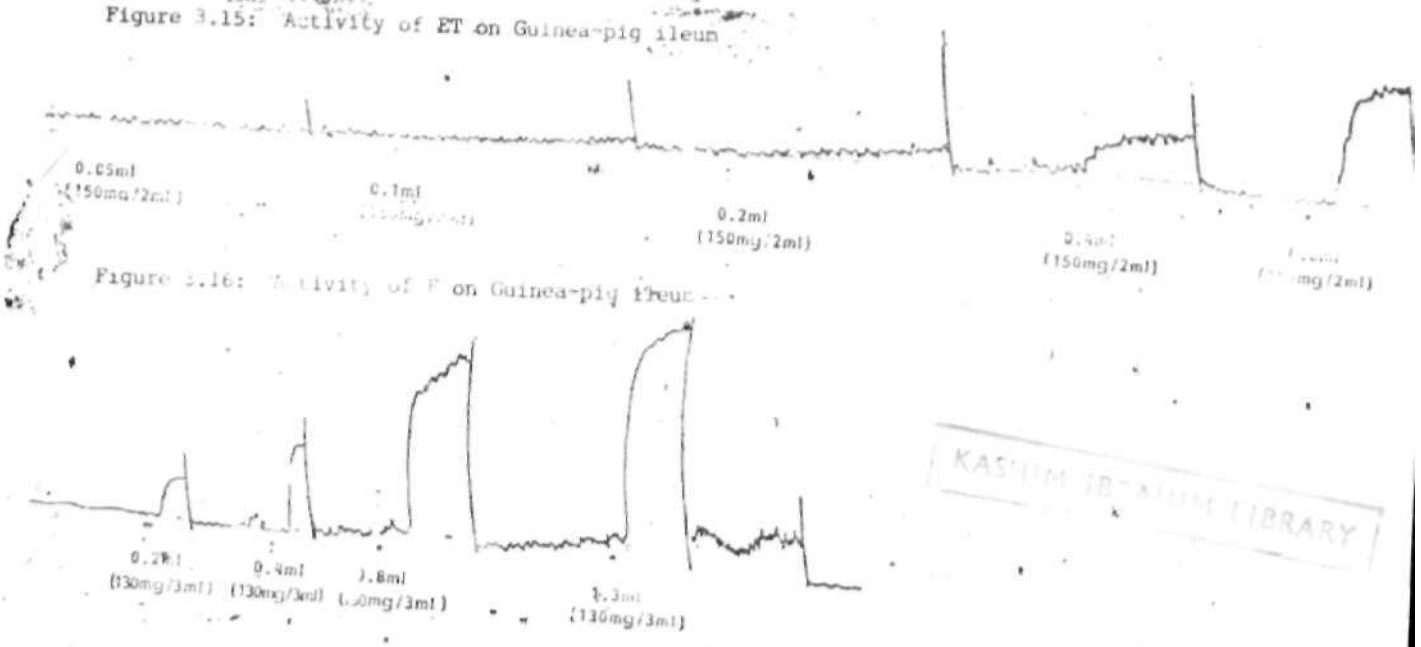
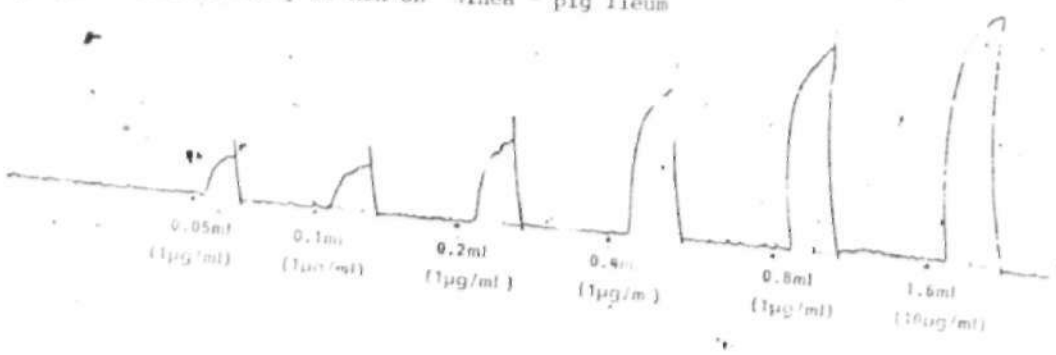


Figure 3.16: Activity of F on Guinea-pig ileum

Figure 3.17: Activity of ACh on Guinea - pig Ileum



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Table 3.4: Dose, Log-dose and Corresponding responses of ACh. ET and F on Guinea-pig ileum

Acetylcholine(ACh)	Dose (ug)	0.050 0.100 0.200 0.400 0.800 1.600
	Log-dose(-2)	0.699 1.000 1.301 1.602 1.903 2.204
	Response(%)	0.152 0.2000.333 0.576 0.803 1.000
Crude Ethanolic Extract(ET)	Dose(mg)	3.750 7.500 15.00 30.00 60.00
	Log-dose	0.570 0.880 1.180 1.480 1.780
	Response(%)	0.000 0.030 0.076 0.182 0.485
Purified Ethanolic Extract (E)	Dose(mg)	8.670 17.33 34.67 56.33
	Loge-dose	0.940 1.240 1.540 1.750
	Response(%)	0.167 0.349 0.712 0.849

The maximum response produced by acetylcholine has been taken as 100% response and all other responses including those produced by the graded doses of both ET and E are calculated as percentages of this. From Table 3.4 it can be observed that it reguired about 30mg of ET and 8.67mg of E to produce comparable response to that produced by just 0.05ug of ACh. indicating that acetylcholine is more potent than E which in turn is slightly more than thrice as potent as ET.

The log dose-response curves, Figure 3.18 (for Ach) and Figure 3.19 (for E and ET) have shown the usual sigmoid nature. Differences in potency are indicated by the slopes of the graphs, with Ach graph more steep. If the graded doses of ACh, ET, and E are converted to the same scale the

Fig. 3.18: Log Dose-Response Curve for Acetylcholine on Guinea - Pig Ileum (No points, however, to justify the dotted lines)

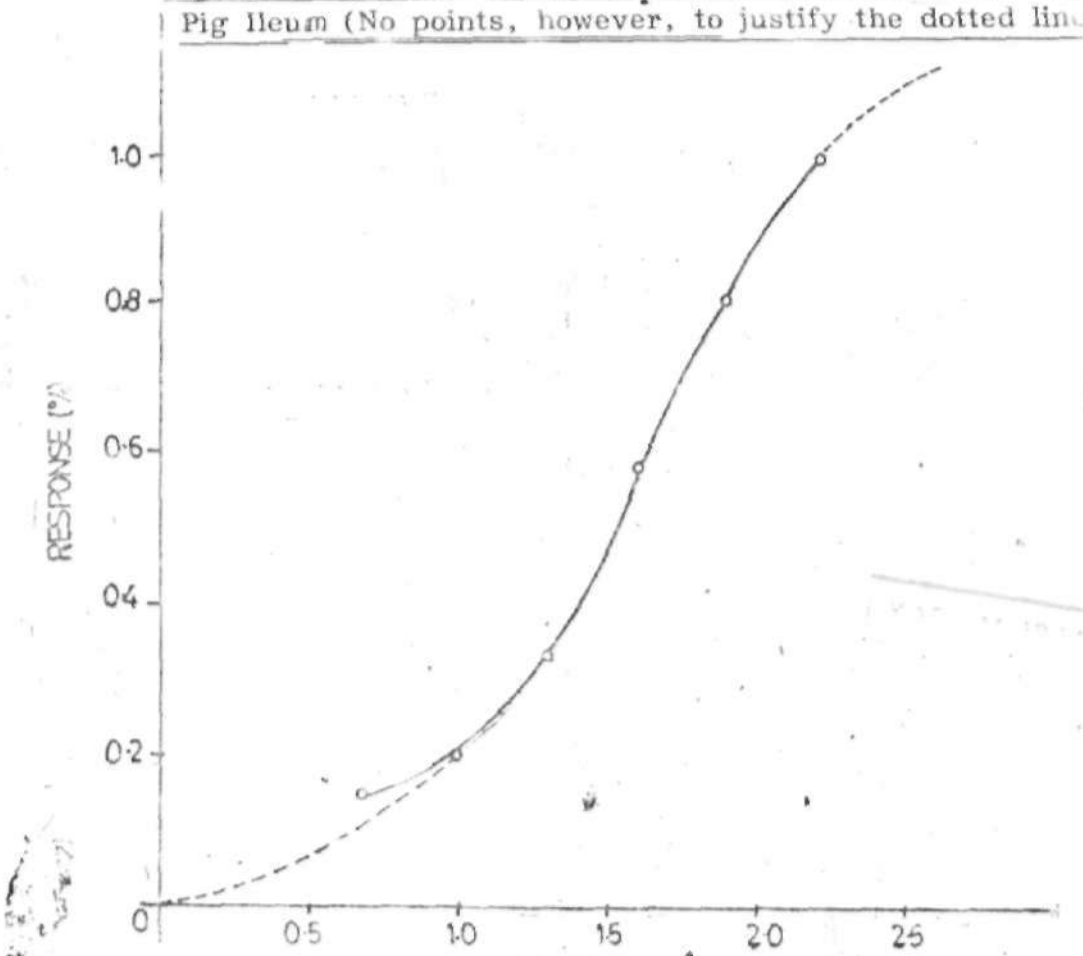
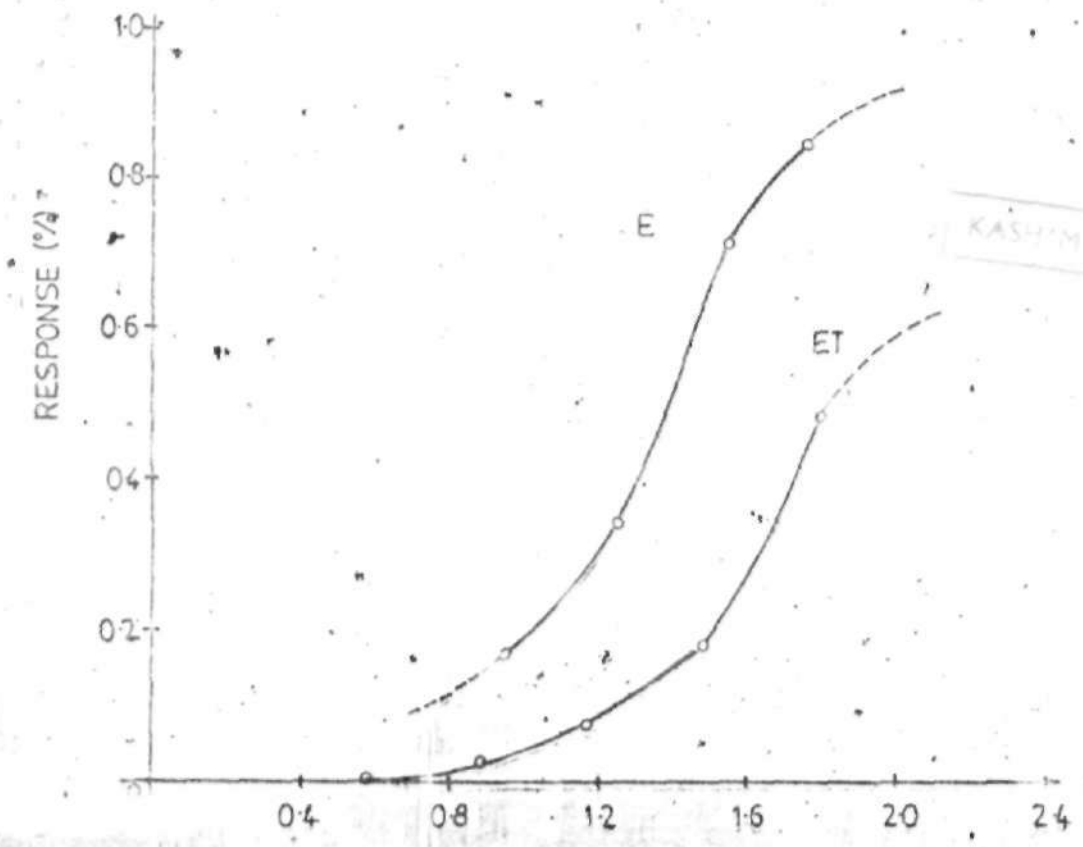


Fig. 3.19: Log Dose - Response Curves for Samples ET and E on Guinea - Pig Ileum (No points, however, to justify dotted lines)



steepness of Ach graph will be more and thus difference in potency would have been much more pronounced.

In vivo (Antifertility) Studies on Female Albino Rats

The antifertility effect of the extract ET with dose, number of implantation sites and percentage of rats with implantation sites on day 10 of pregnancy are shown in Table 3.5.

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Table 3.5: Effect of ET on implantation in female albino rats when fed orally from days 1 to 5 of pregnancy (5 animals were used in each group)

Group	Dose (mg/Kg)	Numberof rats with implantation sites	Number of implantation sites in individual rats	% of rats with implantation sites
Vehicle Control A	-	5	10, 9, 9, 10, 12	100
ET Extract B	40	4	0, 4, 5, 5, 7	80
C	80	1	0, 0, 0, 0, 2	20

The results in Table 3.5 clearly showed that ET extract at 80mg dose was more effective than at 40mg dose pointing out that anti implantation effect due to this extract was dose-dependent. In the animals treated with 80mg dose of the extract, on laparotomy, the uterine horns showed not only reduced number of implantation sites but also of smaller sizes when compared with those of the vehicle control animals.

CONCLUSION

This research work has been set out to ascertain the traditional claim of the use of the plant *Striga senegalensis* in curing stomach-ache in domestic animals (especially goats and sheep) and also in preventing conception in women when the plant material is orally administered.The interest was then to carry out phytochemical screening in order to isolate and characterize the active component(s) and also to investigate the antifertility/anti-implantation effects of the compounds present in the flowers on female albino rats.

A number of chemical substances cause spasm of the smooth muscle of the guinea-pig ileum and these spasmogens of smooth muscles include acetylcholine, histamine, barium chloride and oxytocin. Acetylcholine has important actions at so many sites in the body and the muscarinic receptors in smooth muscles are very similar whether they are in the intestinal smooth muscle, the uterus, or even in the heart. Although these receptors have similar properties, as is usually indicated by sensitivity to agonist and antagonist, this does not necessarily mean that the physiological effects produced by activating the receptors are the same. For example, the action of acetylcholine on the muscarinic receptors in the intestinal smooth muscle and the uterus is to depolarise the muscle and cause it to contract, whereas in the heart it causes hyperpolarisation and reduced contractility. It is

therefore evident that the muscarinic receptors are in some way coupled to a variety of effector mechanisms that initiate the physiological responses that are produced. Although the ethanolic extract of the flowers of the plant has contracted the isolated guinea-pig ileum as does acetylcholine, it may not be surprising that the active principle(s) in the extract may as well be acting on the acetylcholine receptors (muscarinic or nicotinic),or it may imitate histamine in causing the observed contraction, or still it may have a direct effect on the musculature. Histamine contracts the smooth muscle of the guinea-pig ileum and uterus of most species but relaxes the rat uterus and has little effect in humans. Since histamine contracts the guinea-pig ileum comparable to acetylcholine, more studies on the ethanolic extract should be carried out at least for the purpose of identifying the particular receptors that are being stimulated.

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The observed anti-implantation activity of the ethanolic extract in female albino rats may justify the traditional use of this plant in preventing conception. The traditional claim, however, is that the plant material is normally used about 3 months after pregnancy which means after implantation of the zygote might have taken place. To prevent implantation simply means either to expel the zygote from the uterus or to make the endometrium unsuitable for the zygote to be implanted. Abortion may simply mean the expulsion of the foetus from the womb at the early stages of pregnancy and once the pregnancy reached a certain stage abortion ill

normally be very dangerous. Strictly, therefore, there is a distinction between abortifacient activity and anti-implantation activity and to conclude that this plant is an abortifacient drug, more studies, particularly abortifacient activity tests, should be carried out.

On the other face of it, oxytocic activity may be investigated in this plant material since this may be extrapolated from the contraction activity of the plant material on the guinea-pig ileum. The effects of oxytocin in the uterus are quite complex and differ among the various laboratory animals and man. It contracts human, guinea-pig, rabbit and rat uteri. Moreover the responses on the human uterus vary in non-pregnancy and pregnant states and also during the various stages of pregnancy. The mode of action of oxytocin is not well known but it is known that it elicits its contractile action by acting directly on the uterine musculature. It is therefore of vital importance to establish whether this plant material is acting as a neurotropic spasmogen like acetylcholine or a musculotropic spasmogen like oxytocin.

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