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Isolation and Aphrodisiac Screening of the Fruits of *Durio zibenthinus* Linn.

[1]P. Venkatesh, [1]K. Hariprasath, [2]V. Soumya, [2]M. Prince Francis and [2]S. Sankar
[1]Department of Pharmaceutical Chemistry,
Sir C.R. Reddy College of Pharmaceutical Sciences,
Eluru, Andhra Pradesh-534007, India
[2]Department of Pharmaceutical Chemistry, J.S.S. College of Pharmacy,
Rocklands, Ooty, Tamil Nadu-643001, India

Abstract: Aim of the study is to isolate, identify and evaluate the biologically active constituents present in the fruits of *Durio zibenthinus* Linn. The fresh fruit pulp of *Durio zibenthinus* Linn. were successively extracted by using various solvents like petroleum ether, chloroform, ethyl acetate and aqueous alcohol. The petroleum ether extract of the dried fruit pulp of the plant were subjected to isolation of phytoconsituents by counter current extraction and isolated compounds ($-Sitosterol and 3-$-hydroxy-21-Normethyl-19-vinylidenylursane) were partially characterized by using ^{1}H NMR, ^{13}C NMR and Mass spectroscopy. The petroleum ether extract and isolated compound (3-$-hydroxy-21-Normethyl-19-vinylidenylursane) were screened for aphrodisiac activity. The sexually active male mice were administered, the test or the solvent control doses, daily, orally, in their respective concentrations. The activities of male mice in each group were recorded individually for 60 min after 30 min of the drug administration on 1st, 7th and 14th day treatment including sperm count. In conclusion, out of the four extracts of the fruits of *Durio zibenthinus* Linn., Petroleum ether extract was subjected to isolation. From the above, two compounds were isolated. The 400 mg kg^{-1} b.wt. dose of petroleum ether showed significant aphrodisiac activity than all other treated dose of isolated compound (3-$-hydroxy-21-Normethyl-19-vinylidenylursane). Hence, this reveals that most herbs rely for their effects on variety of constituents. Further research is needed to identify biologically active constituents for fertility enhancing activity.

Key words: *Durio zibenthinus* Linn., isolation, ^{1}H NMR, ^{13}C NMR, Mass, aphrodisiac

INTRODUCTION

Natural products are powerful biochemical tools; which serve as Path Finders for molecular biology and chemistry and in the investigation of cellular function (Lixin and Arnold, 2005). The family Bombacaceae is best known for showy flowers and woody or thin-shelled pods filled with small seeds and silky or cotton like fiber. The durian, *Durio zibenthinus* L., is one member that differs radically in having large seeds surrounded by

Corresponding Author: Venkatesh Palaniyappan, Department of Pharmaceutical Chemistry, Sir C.R. Reddy College of Pharmaceutical Sciences, Shanthi Nagar, Eluru, West Godavari District, Andhra Pradesh-534007, India

Fig. 1: *Durio zibenthinus* tree

Fig. 2: *Durio zibenthinus* fruit

fleshy arils. Apart from variants of the word durian in native dialects, there are few other vernacular names, though the notorious odor has given rise to the unflattering terms, civet cat tree (Fig. 1) and civet fruit (Fig. 2) in India. Nevertheless the durian is the most important native fruit of South-Eastern Asia and neighboring islands. The fruits are ovoid or ovoid-oblong to nearly round, 18 lbs (8 kg) in weight and some fruits split into 5 segments, others do not split, but all fall to the ground when mature (Morton, 1987).

Earlier study reported that $-galactosidase were isolated from durian (Tanboly, 2001), forty-three sulphur-containing constituents were found in a pentane extract of the durian fruit (Naf and Velluz, 1998), 63 constituents were identified, comprising 30 esters, 16 sulphur containing compounds, 5 ketones, 8 alcohols and 4 miscellaneous compounds (Wong and Tie, 2006) and ethyl 2-methylbutanoate was found to have the highest odor impact among the non-sulfurous odorants in durian (Weenen *et al*., 1996).

The term infertility is used to describe a couple who has been unable to conceive naturally after two years of unprotected intercourse (Miller *et al*., 2007). Traditionally the fruits of *Durio zibenthinus* are being used by people all over the world for their fertility enhancing activity. Hence an attempt to evaluate the plant for its influence on copulatory behavior, sperm count and sperm motility in the male species of Swiss mice is being considered as worthwhile investigative undertaking.

MATERIALS AND METHODS

Collection and Identification of Plant

The plant fruits were collected in the month of August 2008 from the State Horticulture Farm, *Burliar*, The Niligiris, Tamil Nadu, India. The plant was authenticated by comparing it with authentic specimen at the Botanical survey of India, Coimbatore, Tamil Nadu, India.

Extraction of the Fruits of *Durio zibenthinus* Linn.

The fresh fruits of *Durio zibenthinus* Linn. which were made free from mud and other impurities and dried in shade. The dried fruit pulp was then powdered and subjected to successive hot extraction using non polar to polar solvents. The solvents used for successive extracts were petroleum ether, chloroform, ethyl acetate and aqueous alcohol.

To 380 g of dried fruit pulp powder, 1.5 L of petroleum ether was added and extracted at 60°C for 24 h in a Soxhlet apparatus. After 24 h the petroleum ether fraction was filtered through a Whattman filter paper and the marc was again successively extracted using chloroform, ethyl acetate and aqueous alcohol respectively by the same procedure. All the extracts were then concentrated and dried under reduced pressure with a controlled temperature (40-55°C) using the rotary evaporator (Kokate *et al*., 1997).

Isolation of Phytoconstituents of *Durio zibenthinus* Linn. Extracts

The petroleum ether extract was subjected to isolation of phytoconstituents by counter current method using various solvents like acetone, chloroform, methanol and hexane.

In vivo Screening Studies of the Petroleum Ether Extract and Isolated Compound (3-$-hydroxy-21-Normethyl-19-vinylidenylursane)

The sexually active male Swiss mice (25-35 g) were grouped separately and divided into 5 groups, each group consisting of 6 animals. Group I received solvent control (0.3% CMC), group II received petroleum ether extract 200 mg kg^{-1} b.wt. in 0.3% CMC, group III received petroleum ether extract 400 mg kg^{-1} b.wt. in 0.3% CMC, group IV received isolated compound (3-$-hydroxy-21-Normethyl-19-vinylidenylursane) 20 mg kg^{-1} b.wt. in 0.3% CMC and group V received isolated compound (3 $-hydroxy-21-Normethyl-19-vinylidenylursane) 40 mg kg^{-1} b.wt. in 0.3% CMC, daily, orally using oral catheter. Sexual behavior was observed in a dim light at day time in specially designed cages having glass on all sides and measuring 50×30×30 cm (Fig. 3). The male experimental mice were transferred to the cage and

Fig. 3: Male mice showing mounting behavior

the female mice in oestrous phase were introduced with males. The first 15 min were considered as acclimatization period (Ageel *et al*., 1994). The activities of male mice in each group were recorded individually for 60 min after 30 min of the drug administration on 1st, 7th and 14th day treatments. The parameters viz. mounting (Subramoniam *et al*., 1997), intromission (Suresh-Kumar *et al*., 2000), sperm count and sperm motility were observed.

Sperm Count

One milliliter of diluting fluid (sodium bicarbonate 5 g and formalin neutral 1 mL in 100 mL of distilled water) was measured into a clean test tube. To this 0.1 mL of seminal fluid collected from epididymis using forced extraction was added, which yields a 1:10 dilution. Neubauer's chamber (0.0025 mm^2, Tiefe depth profounder 0.100 mm, Superior Marienfield Germany) was filled with the diluted seminal fluid. The chamber was left on the bench for 2 min. This allows the immobilized sperms to settle down. Then the numbers of sperms in the four corner squares covering 4 mm^2 were counted under the high power objective (40x) (Mukherjee, 1997). Sperm count was calculated as per the formula:

$$\text{Sperm count (mL}^{-1}) = \text{Sperm count} \times 10 \times 1000/4 \times 0.1$$

Sperm Motility

Single Cauda of the epididymis was punctured with a 21 gauge hypodermic needle. Fluid content from the epididymal lumen was collected. A small drop of fluid was placed in pre-warmed microscopic slide, approximately at body temperature. The drop was covered with a cover slip by the WET MOUNTING TECHNIQUE. The cover slip was rimmed with vaseline to avoid drying. The slide was examined under the high power objective (40X) with reduced illumination. Several fields were scanned until a total of at least 200 sperms have been observed. The percentage of sperm showing actual progressive motion was calculated. Sperm motility was expressed in terms of the percentage of sperms which were active (Mukherjee, 1997).

RESULTS

Isolation of Phytoconstituents of *Durio zibenthinus* Linn. Extracts

The phytoconstituents present in the petroleum ether extract of *Durio zibenthinus* Linn. were isolated by counter current extraction and were partially characterized by ^{1}H NMR, ^{13}C NMR and Mass are shown in the Fig. 4-5. Compound I (Fig. 4 and 5, the ^{1}H NMR spectrum displayed signals for two tertiary methyl groups at * 0.68 (s, 3H) and 1.01 (s, 3H) and three secondary methyl groups at * 0.93 (d, 3H), 0.81 (d, 3H) and 0.83 (d, 3H) and primary methyl group at * 0.85 (m, 3H). The broad singlet at * 5.35 was attributed to H-6 and the hydroxyl methane proton signal at * 3.55 was attributed to H-3 proton based on biogenetic considerations. From Fig. 7 the molecular ion peak was found to be 415.00, the above data and knowledge of known sterols the compound I was found to be $-Sitosterol).

Compound II (Fig. 10), the ^{1}H NMR spectrum showed signals for six C-methyl singlet's at * 0.76, 0.78, 0.88, 0.94, 0.98 and 1.01, an allylic methyl group at * 1.68, Fig. 11 showed signals for a vinylidene grouping exhibited by a pair of doublet signals at * 4.56 and 4.68 each integrating for one proton and a hydroxy methine group at * 3.19 (dd) which may be placed between a tetrasubstituted sp^3 carbon atom and a methylene grouping. From a biogenetic point of view, the C-3 hydroxy group was assigned equatorial $-orientation, it is

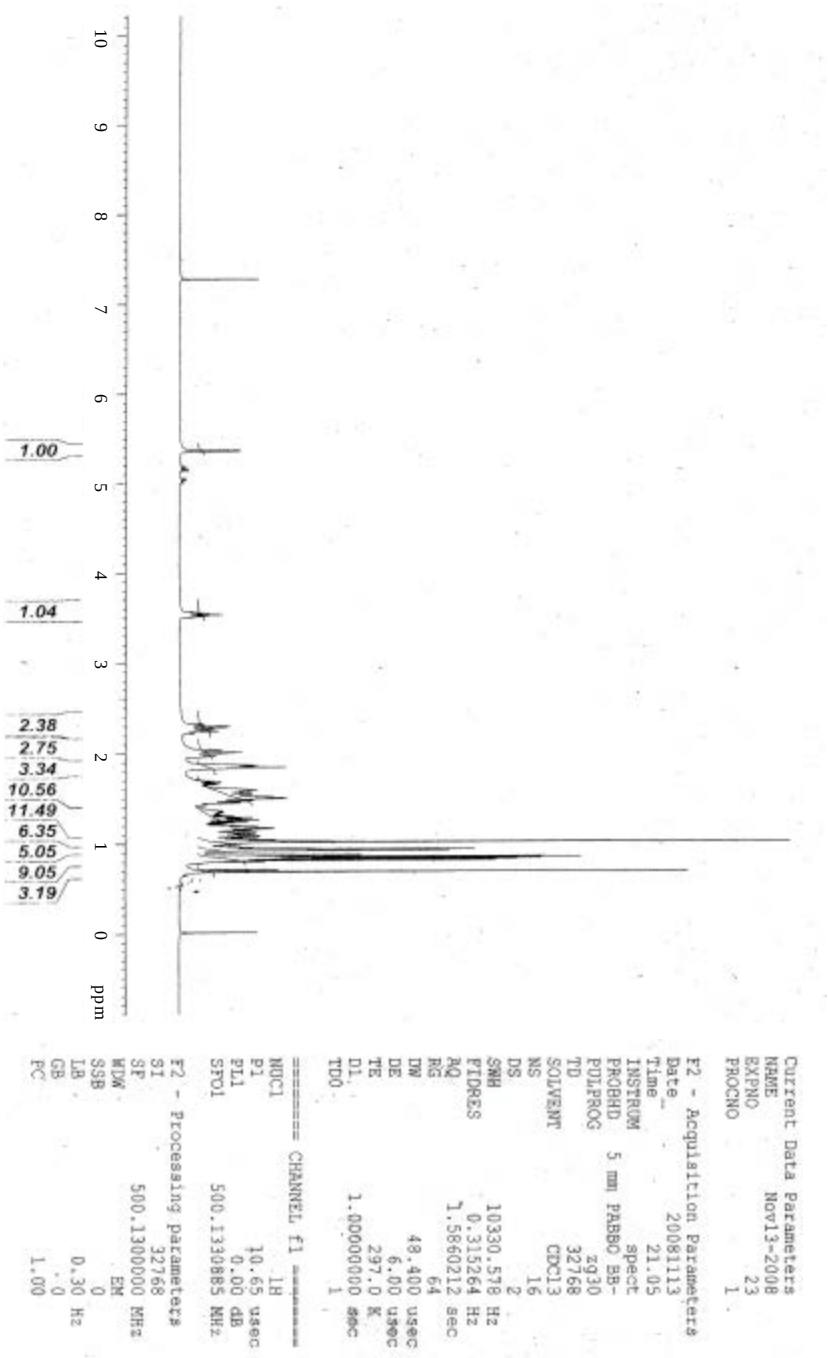

Fig. 4: ^{1}H NMR of cmpd I isolated compound I

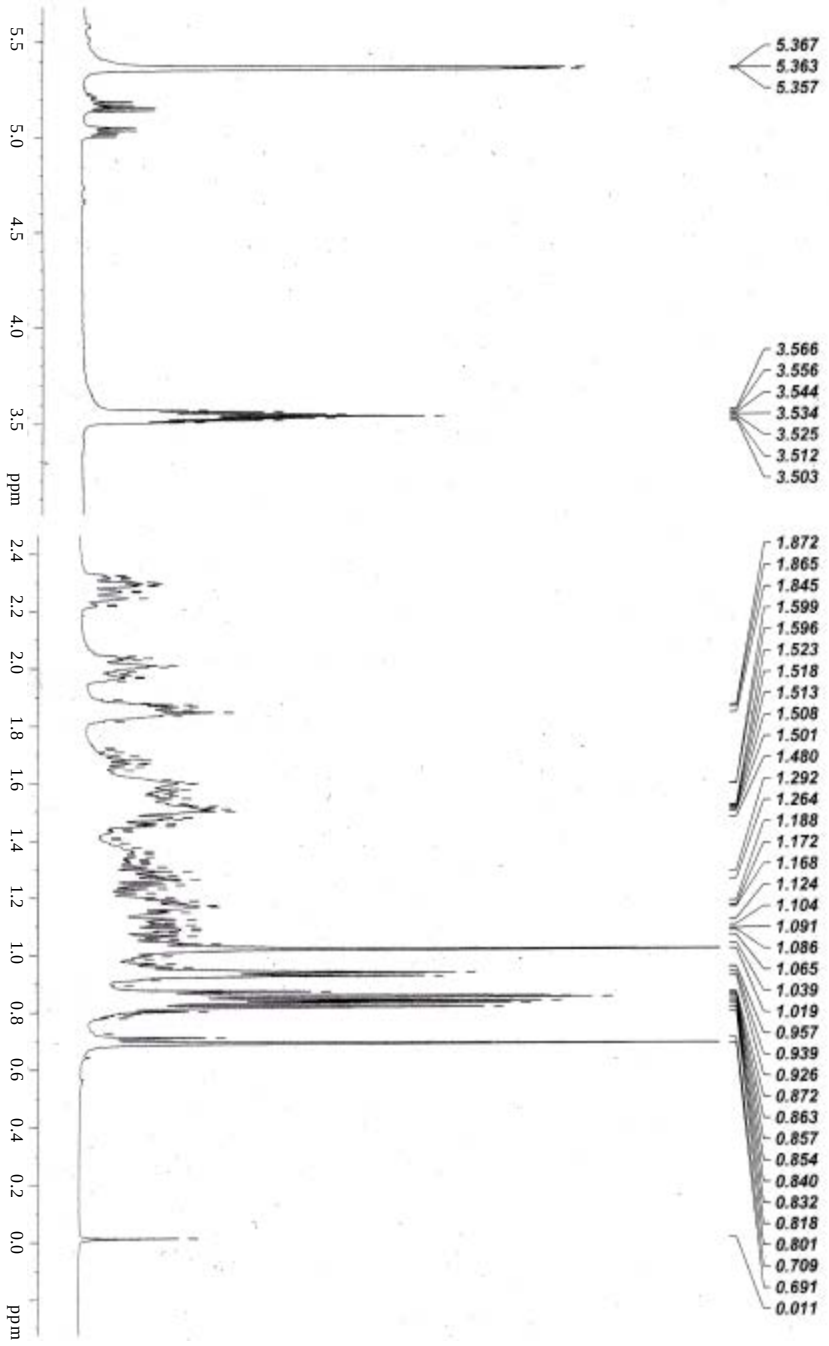

Fig. 5: ^{1}H NMR of isolated compound I

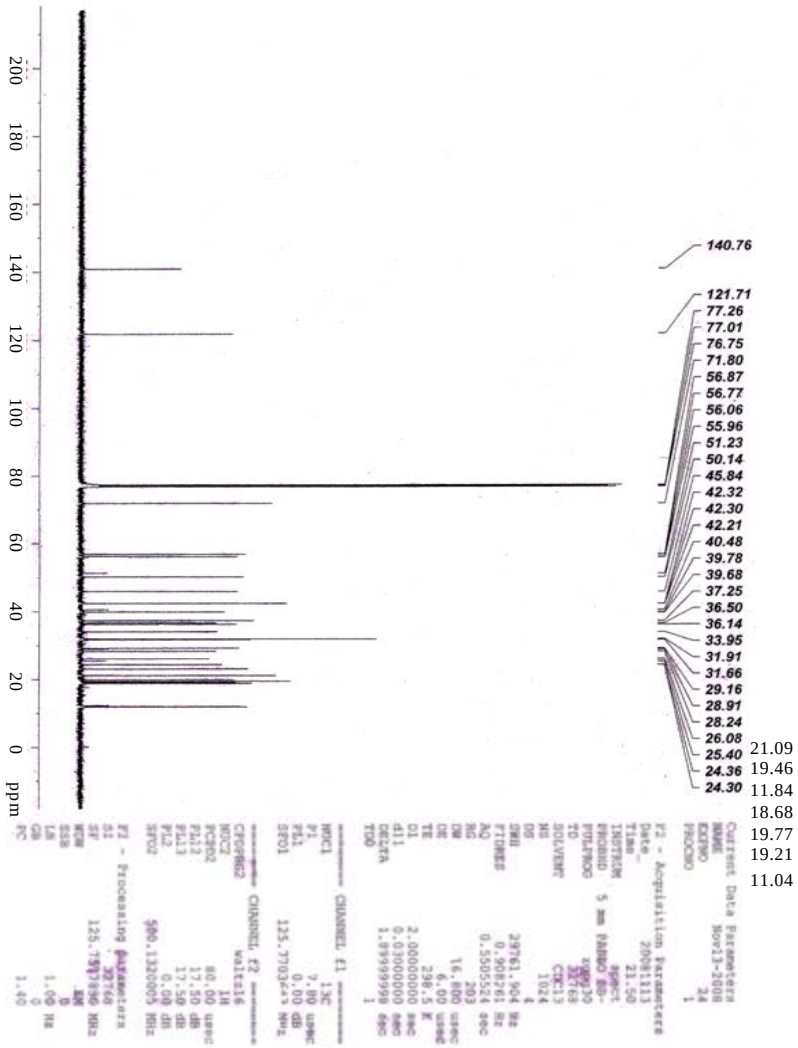

Fig. 6: ^{13}C NMR of isolated compound I

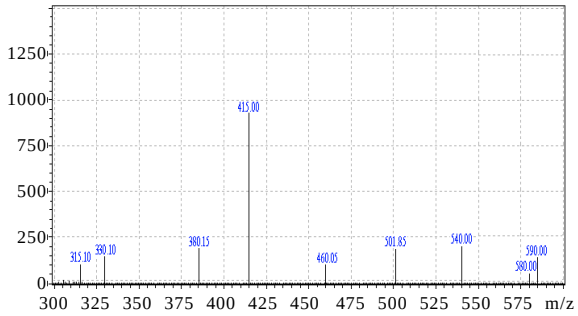

Fig. 7: Mass of isolated compound I

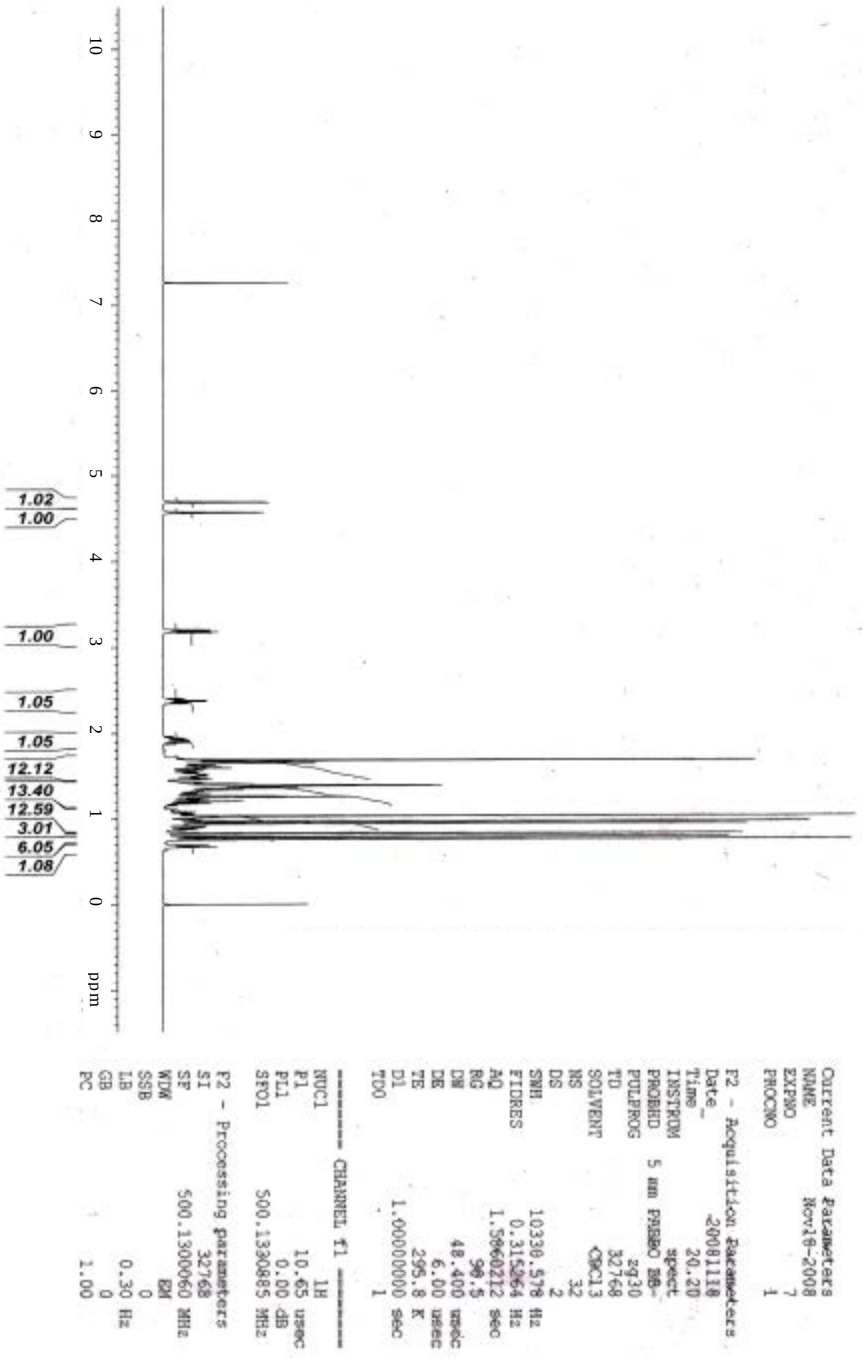

Fig. 8: ^{1}H NMR of isolated compound II

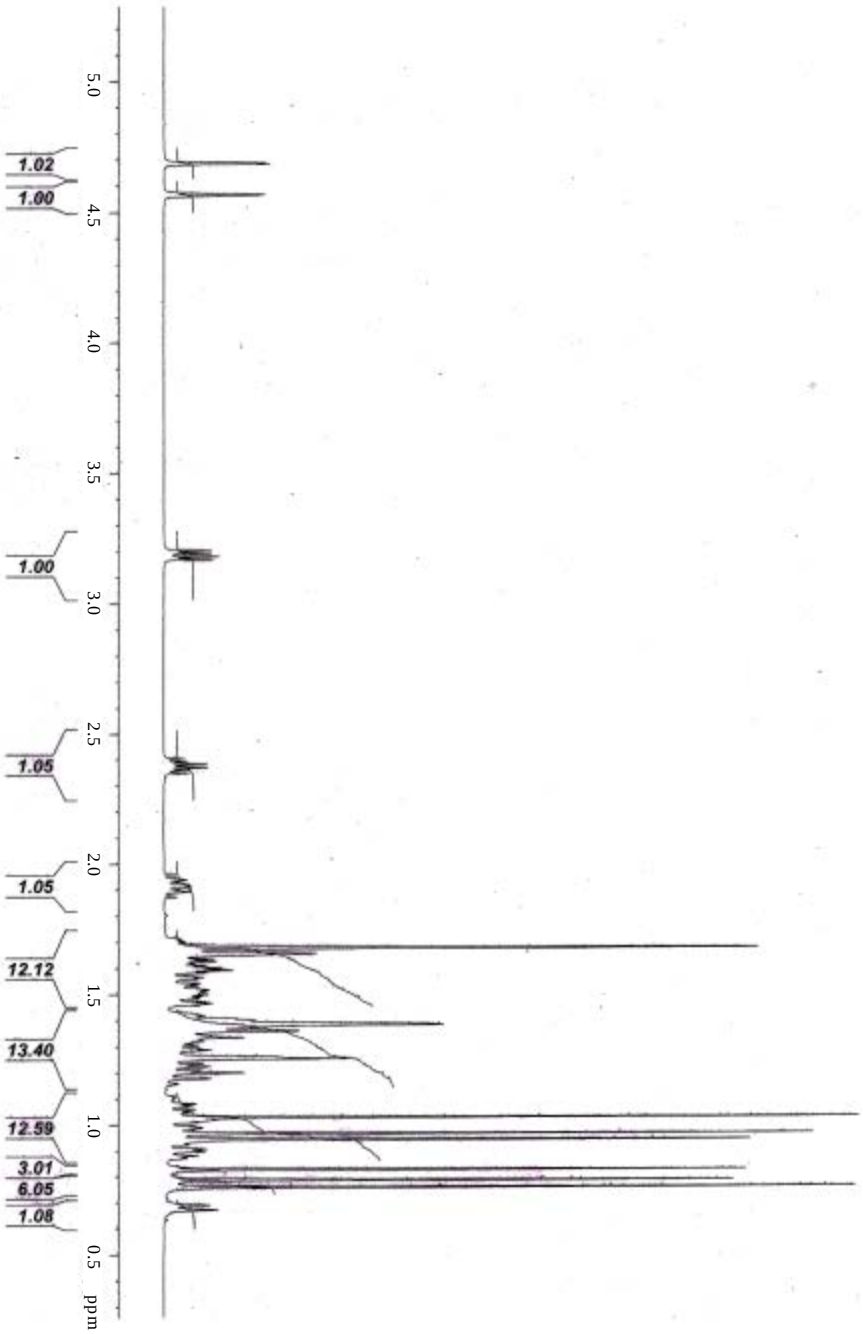

Fig. 9: ^{1}H NMR of isolated compound II

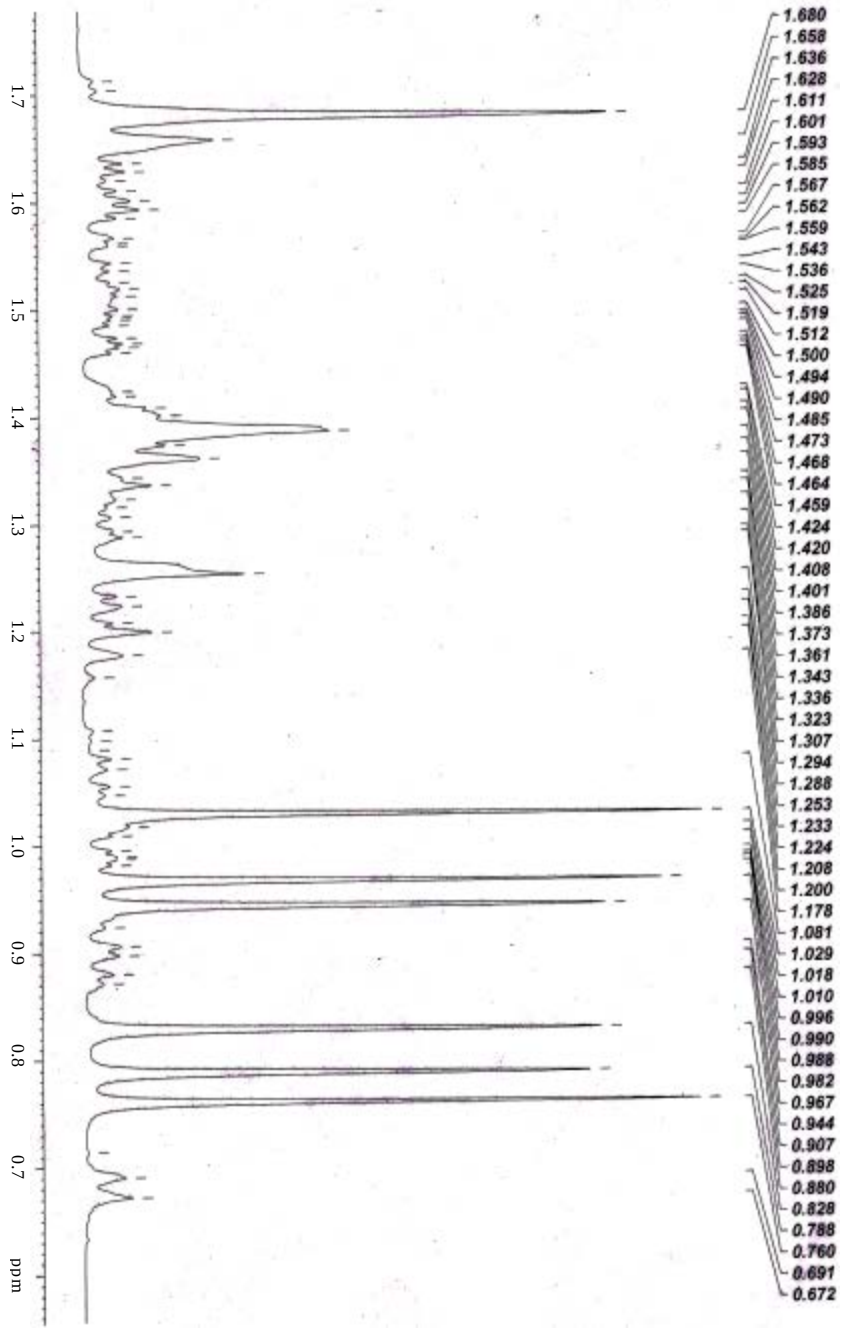

Fig. 10: ^{1}H NMR of isolated compound II

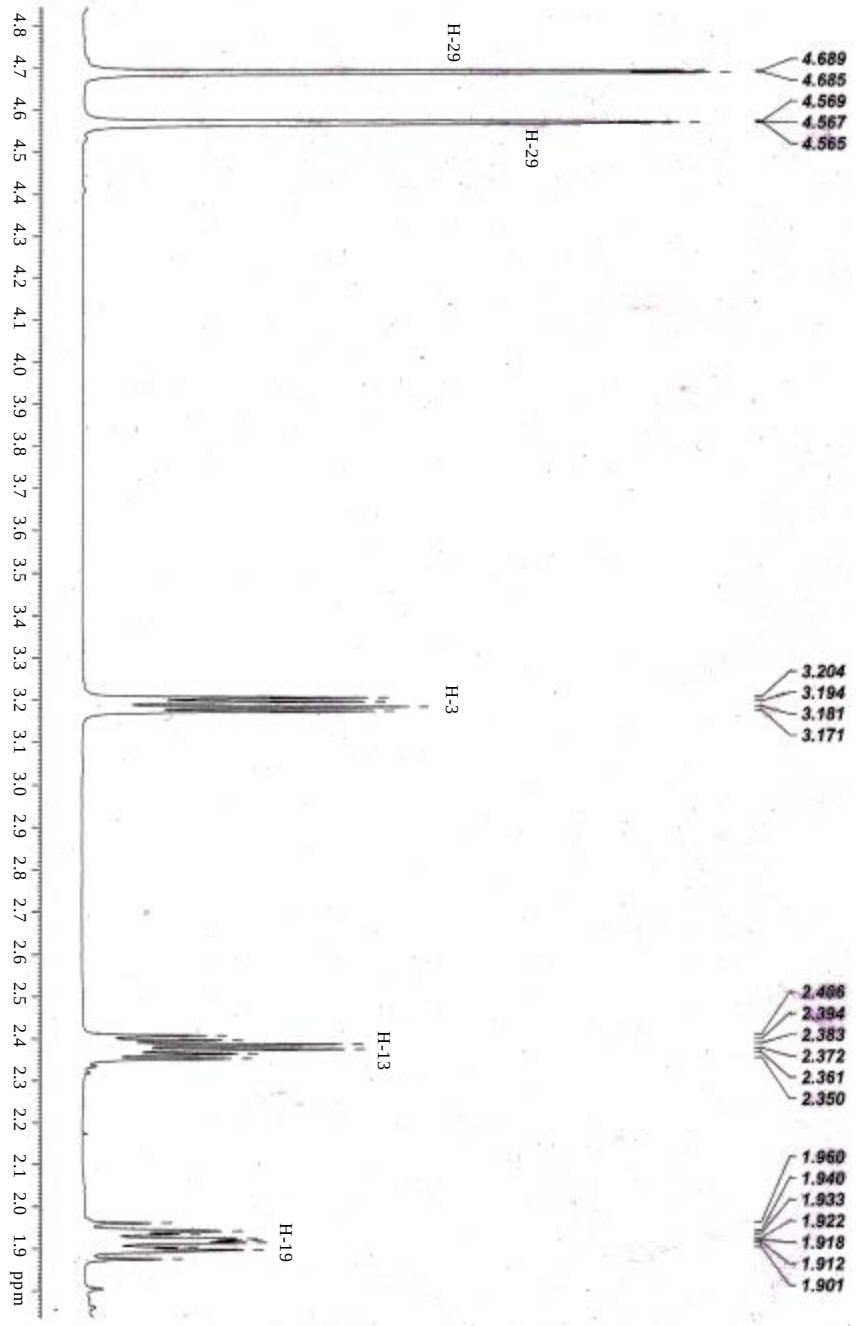

Fig. 11: ^{1}H NMR of isolated compound II

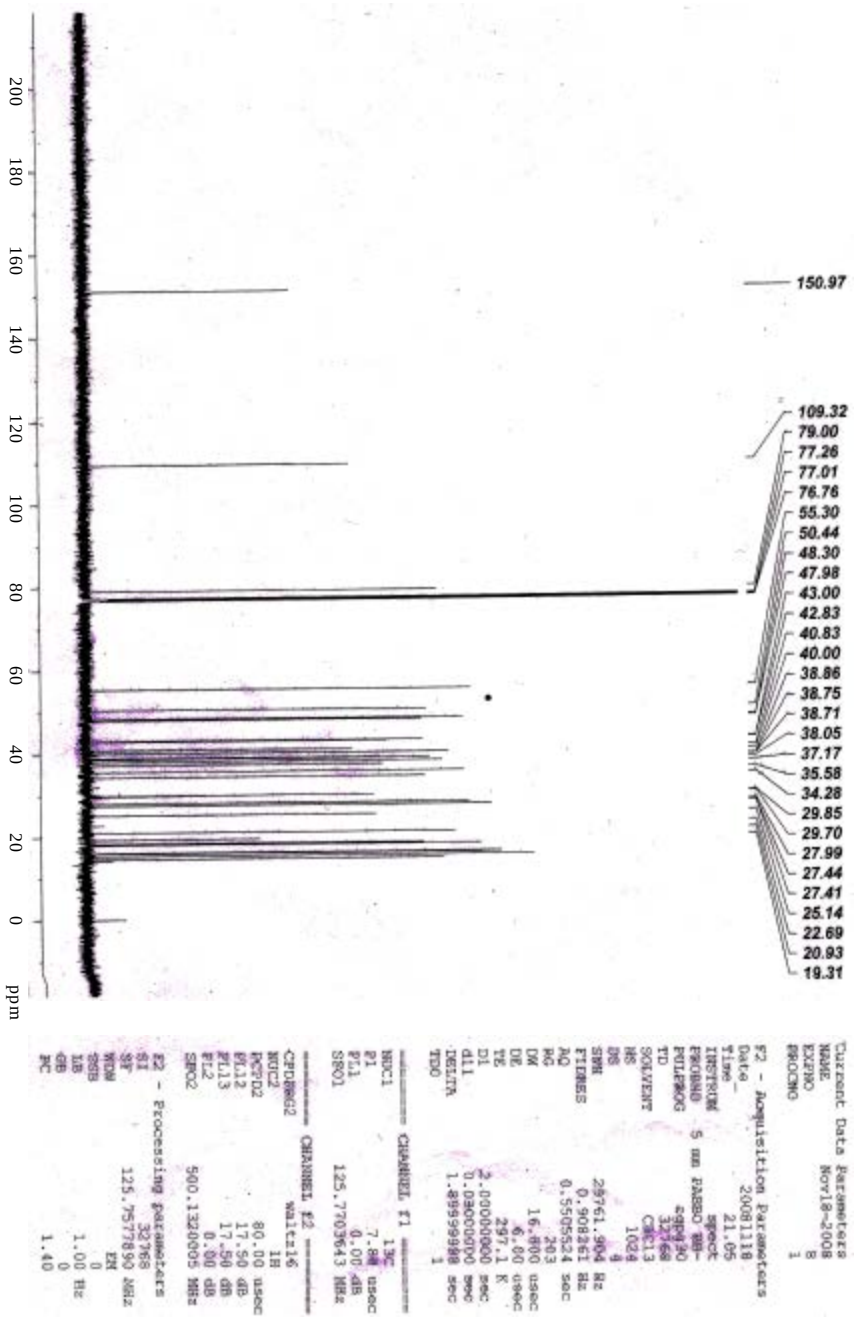

Fig. 12: ^{13}C NMR of isolated compound II

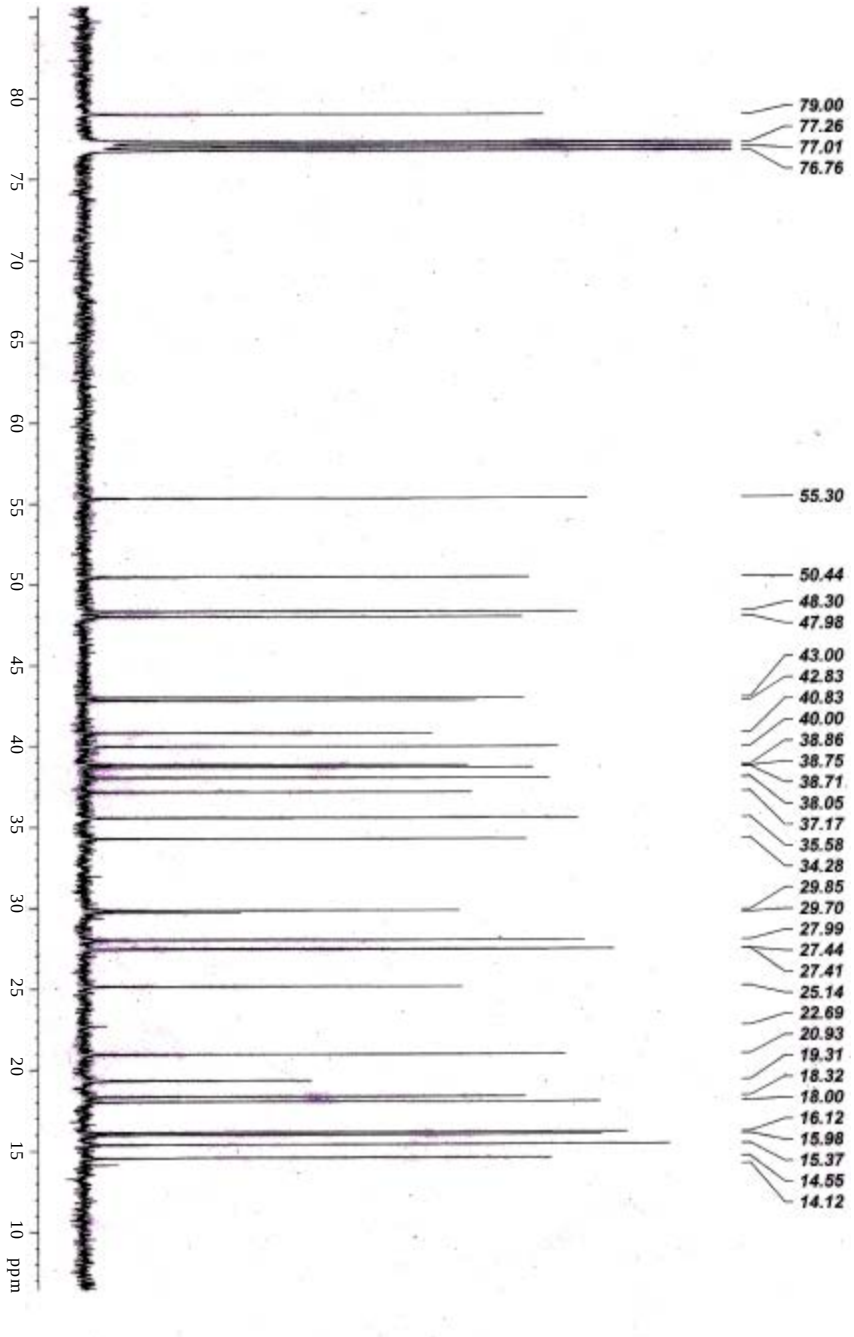

Fig. 13: ^{13}C NMR of isolated compound II

common feature in Triterpenoids. The presence of C-3 hydroxy group was supported by C^{13}NMR (Fig. 12 and 13) signal at * 79.00. The signals at * 109.32 and 150.97 are due to unsaturated carbon atoms between C_{20} and C_{30}. From Fig. 8 and 9 the signals at * 2.38 is due to H-13$ and at * 1.9 is due to H-19.

From Fig. 14 the molecular ion peak was found to be 440.85, the above data suggested the compound II was (3-$-hydroxy-21-Normethyl-19-vinylidenylursane).

***In vivo* Screening Studies of the Petroleum Ether Extract and Isolated Compound (3-$-hydroxy-21-Normethyl-19-vinylidenylursane)**

The parameters viz., mounting (Fig. 3), intromission, sperm count and sperm motility were observed and results are shown in the Table 1-5.

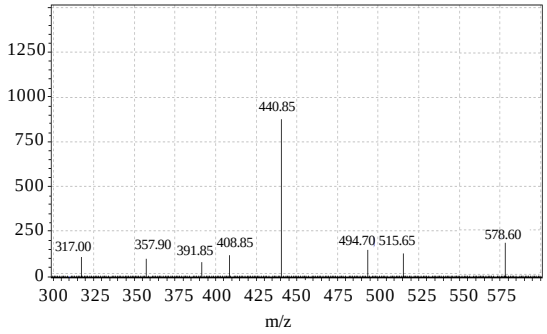

Fig. 14: Mass of the isolated compound II

Table 1: Control

	Day		
Parameters	1st	7th	14th
Mount frequency	3.33±0.21	4.50±0.34	3.83±0.40
Mount latency (sec)	586.67±3.33	531.67±1.67	476.67±2.11
Intromission frequency	2.83±0.31	2.67±0.33	3.00±0.67
Intromission latency (sec)	755.00±2.24	665.00±2.24	780.00±2.58
Sperm count (millions mL^{-1})	-	-	1.038±0.52
Sperm motility (%)	-	-	58.80±0.89

Route of administration: Oral, Values are Mean±SEM of 6 animals, Statistical significance: d = ns = p>0.05, c = p<0.05, b = p<0.01, a = p<0.001 as compared to the solvent control group, Bonferroni compare selected pairs of columns

Table 2: Petroleum ether extract 200 mg kg^{-1} b.wt. in 0.3% CMC

	Day		
Parameters	1st	7th	14th
Mount frequency	7.17±0.31^{a}	11.08±0.55^{a}	12.50±0.60^{a}
Mount latency (sec)	310.00±2.58^{a}	280.04±1.77^{a}	210.14±1.89^{a}
Intromission frequency	4.56±0.15^{a}	7.20±0.20^{a}	10.12±0.38^{a}
Intromission latency (sec)	380.14±1.71^{a}	280.36±2.57^{a}	210.42±0.85^{a}
Sperm count (millions mL^{-1})	-	-	1.075±0.20^{d}
Sperm motility (%)	-	-	60.24±0.45^{d}

Route of administration: Oral, Values are Mean±SEM of 6 animals, Statistical significance: d = ns = p>0.05, c = p<0.05, b = p<0.01, a = p<0.001 as compared to the solvent control group, Bonferroni compare selected pairs of columns

Table 3: Petroleum ether extract 400 mg kg^{-1} b.wt. in 0.3% CMC

	Day		
Parameters	1st	7th	14th
Mount frequency	9.55±0.32^{a}	12.74±0.52^{a}	16.64±0.91^{a}
Mount latency (sec)	290.22±1.68^{a}	180.28±0.79^{a}	124.16±2.10^{a}
Intromission frequency	6.24±0.36^{a}	10.64±0.51^{a}	13.24±0.51^{a}
Intromission latency (sec)	274.16±2.54^{a}	168.54±1.49^{a}	154.02±0.81^{a}
Sperm count (millions mL^{-1})	-	-	1.366±0.53^{a}
Sperm motility (%)	-	-	62.32±0.47^{d}

Route of administration: Oral, Values are Mean±SEM of 6 animals, Statistical significance: d = ns = p>0.05, c = p<0.05, b = p<0.01, a = p<0.001 as compared to the solvent control group, Bonferroni compare selected pairs of columns

Table 4: Isolated compound (3-$-hydroxy-21-Normethyl-19-vinylidenylursane) 20 mg kg^{-1} b.wt. in 0.3% CMC

	Day		
Parameters	1st	7th	14th
Mount frequency	4.89±0.36^{d}	6.84±0.42^{c}	8.12±0.19^{a}
Mount latency (sec)	480.12±1.04^{a}	380.14±0.49^{a}	280.65±1.31^{a}
Intromission frequency	3.98±0.05^{b}	5.98±0.52^{a}	6.54±0.59^{a}
Intromission latency (sec)	480.50±0.78^{a}	340.02±0.40^{a}	290.50±1.02^{a}
Sperm count (millions mL^{-1})	-	-	1.032±0.31^{d}
Sperm motility (%)	-	-	58.74±1.17^{d}

Route of administration: Oral, Values are Mean±SEM of 6 animals, Statistical significance: d = ns = p>0.05, c = p<0.05, b = p<0.01, a = p<0.001 as compared to the solvent control group, Bonferroni compare selected pairs of columns

Table 5: Isolated compound (3-$-hydroxy-21-Normethyl-19-vinylidenylursane) 40 mg kg^{-1} b.wt. in 0.3% CMC

	Day		
Parameters	1st	7th	14th
Mount frequency	5.89±0.70^{a}	7.04±0.60^{b}	10.14±1.00^{a}
Mount latency (sec)	410.14±1.05^{a}	320.24±0.42^{a}	256.04±0.52^{a}
Intromission frequency	4.00±0.05^{b}	7.02±0.69^{a}	8.04±0.67^{a}
Intromission latency (sec)	374.16±2.10^{a}	290.15±1.00^{a}	246.33±1.00^{a}
Sperm count (millions mL^{-1})	-	-	1.036±0.13^{d}
Sperm motility (%)	-	-	58.94±1.97^{d}

Route of administration: Oral, Values are Mean±SEM of 6 animals, Statistical significance: d = ns = p>0.05, c = p<0.05, b = p<0.01, a = p<0.001 as compared to the solvent control group, Bonferroni compare selected pairs of columns

DISCUSSION

On the first day of the treatment all the treated groups showed increased copulatory sexual behavior in all the experimental animals as revealed by the results. The prolonged treatment of all the treated groups was highly effective to increase the sexual libidity as compared to the solvent control. This indicates that the aphrodisiac activity has been shown by the tested petroleum ether extract and isolated compound (3-$-hydroxy-21-Normethyl-19-vinylidenylursane) at all the tested dose levels. The order of potency for the petroleum ether extract and isolated compound (3-$-hydroxy-21-Normethyl-19-vinylidenylursane) were:

Pet ether extract at 400 mg kg^{-1} b.wt. > Pet ether extract at 200 mg kg^{-1} b.wt. > Isolated compound at 40 mg kg^{-1} b.wt. > Isolated compound at 20 mg kg^{-1} b.wt.

Finally, two compounds were isolated. The petroleum ether extract and isolated compound (3-$-hydroxy-21-Normethyl-19-vinylidenylursane) were screened for aphrodisiac activity. The petroleum ether extract at 400 mg kg^{-1} b.wt. doses showed better aphrodisiac activity than all other treated doses.

In support to the above work, phytochemical investigations have shown the presence of glycoside, saponins, flavanoids and sterols in Durian fruit. It is likely that these steroidal constituent increase the steroidogenesis and elevate androgen levels which results in observed effect (Chauhan and Dixit, 2008). So, the petroleum ether extract showed better activity due to presences of sterols.

Generally sexual behaviours are enhanced by elevated testosterone levels. Drug induced changes in neurotransmitter levels or their action in the cells could also change sexual behavior. In this connection it should be remembered that on ethnomedical practices this herb is also considered as a nervous stimulant (Chopra *et al*., 1956). Investigations are in progress to explore the possible mechanism of action.

CONCLUSION

There can be no doubt that most herbs rely for their effects on a variety constituents and the idea of synergy within and between them. So this plant may also contain other constituents which also possess fertility enhancing activity. Hence, future study on this plant should be in isolation of other constituents which may show fertility enhancing activity. Once a molecule has been isolated which possess activity then its derivatives could be synthesized and QSAR studies can be conducted which gives optimized result for activity (Wolf, 1997).

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REFERENCES

Ageel, A.M., M.W. Islam, O.T. Ginawi and M.A. Al-Yahya, 1994. Evaluation of the aphrodisiac activity of *Listea chinesis* (*Lauraceae*) and *Orchis malculata* (*Orchidaceae*). Phytotheraphy Res., 8: 103-105.

Chauhan, N.S. and V.K. Dixit, 2008. Spermatogenic activity of rhizomes of *Curculigo orchioides* Gaertn in male rats. Int. J. Applied Res. Nat. Prod., 1: 26-31.

Chopra, R., S.L. Nayar and I.C. Chopra, 1956. Glossary of Indian Medicinal Plants. 1st Edn., National Institute of Science Communications, New Delhi, India.

Kokate, C.K., A.P. Purohit and S.B. Gokhale, 1997. Text Book of Pharmacognosy. 7th Edn., Nirali Prakashan, Pune, India.

Lixin, Z. and L. Arnold, 2005. Natural Product Drug Discovery and Therapeutic Medicine. Humana Press, New Jersey.

Miller, J., A.B. Boyden and K.A. Frey, 2007. Infertility. Am. Fam. Phys., 75: 894-856.

Morton, J., 1987. Fruits of warm climates. Durian, 1: 287-291.

Mukherjee, K.L., 1997. Medical Laboratory Techniques, A Procedure Manual for Routine Diagnostic Tests. 2nd Edn., Tata McGraw Hill, USA.

Naf, R. and A. Velluz, 1998. Sulphur compounds and some uncommon Esters in Durian (*Durio zibenthinus* Murr.). Flavour Fragrance J., 11: 295-303.

Subramoniam, A., V. Madhavachandran, S. Rajasekaran and P. Pushpangadam, 1997. Aphrodisiac property of *Trichopus zeylanicus* extract in male mice. J. Ethnopharmacol., 57: 21-27.

Suresh-Kumar, P.K., A. Subramoniam and P. Pushpangadan, 2000. Aphrodisiac activity of Vanda tessellata (Roxb.) Hook. Ex Don extract in male mice. Indian J. Pharmacol., 32: 300-304.

Tanboly, E.E., 2001. The $-galactosidase system of a novel plant from durian seeds (*Durio zibethinus*) I. isolation and partial characterization. Pak. J. Biol. Sci., 4: 1531-1534.

Weenen, H., E. Wim and A. Apriyantono, 1996. Sulfur-Containing Volatiles of Durian Fruits (*Durio zibethinus* Murr.). J. Agric. Food Chem., 44: 3291-3293.

Wolf, E., 1997. Burgers Medicinal Chemistry and Drug Discovery. 5th Edn., Wiley-Inter Science, New York.

Wong, K.C. and D.Y. Tie, 2006. Volatile constituents of durian (*Durio zibethinus* Murr.). Flavour Fragrance J., 10: 79-83.