



CHEMICAL INVESTIGATION AND ANTI-FERTILITY ACTIVITY OF ACACIA FARNESIANA (A MEDICINAL PLANT)

Pratima Singh* and Manoj Kumar Srivastava

*Department of Chemistry, Digvijay Nath P.G. College, Gorakhpur-273001, India.

#Department of Chemistry, Mahatma Gandhi P.G. College, Gorakhpur-273001, India.



*Corresponding Author: Pratima Singh

Department of Chemistry, Digvijay Nath P.G. College, Gorakhpur-273001, India.

Article Received on 10/11/2024

Article Revised on 01/12/2024

Article Accepted on 22/12/2024

ABSTRACT

Fertility is the natural capability of giving life. The contribution of Phytochemistry of modern medicine is immense. In recent years, some important life saving drugs have been obtained from plants. e.g. anti-cancer drugs, **Vincristine** and **Vinblastine** from *Catharanthus roseus*. It is now well realized that systemic chemical and biological evaluation of plants can provide new drugs and new leads of synthesis of novel chemical compounds which would have a better pharmacological profile, lesser toxicity. The plants as Abortifacient and as contraceptive well known to the ancient physicians of India. Various medicinal plants extracts have been tested for their Anti-fertility both in male and female animals. Fertility control is an issue of global public health concern. The present search is aimed to summarize the fertility regulatory of plant *Acacia farnesiana* with its extract and isolated compounds along with animals model used.

KEYWORDS: Pharmacology, anti-fertility, Medicinal plants, Birth control, Contraceptives.

INTRODUCTION

Over population of world is one of the serious problem in the developing countries like INDIA and would be increased day by day. Global search on anti-fertility agent is going on to tackle the problem of population explosion. Many hormonal drugs are available for the purpose but they are not free from side effects. Hence, the search for a suitable products from indigenous medicinal plants is proposed which could be effectively used in the place of pills. *Sesbania sesban* belonging to family leguminosae has been reported as anti-fertility activity^[1-4] of flowers and leaves in albino rats and mice. The flower of *Sesbania sesban* reported as abortifacient^[5] activity and observed anti-implantation^[6] effect in albino rats.

Rising human population throughout the world more particularly in INDIA has detrimental effect on the life supporting system on the earth. Several potential approach for induction of infertility have been investigated over a long period including hormonal, chemical and immunological approaches. The chemical compounds affected testicular function include different groups like steroidal and non-steroidal among them are : Danazol, Depotmexdroxy Progesterone acetate (DMPA), Cyproterone acetate (CPA), Levenogestral, Melatonin. α -Chlorohydrin, Metapiron and Serotonin. These are hazardous for reproductive organs.^[7]

There has been a steady accumulation of information regarding the screening of plants having anti-fertility activities.^[8-16] Therefore, during the present course of investigation *Acacia farnesiana* is chosen for chemical studies. The plant has been found to exhibit mild anti-fertility and implantation activity in rats.

Introduction to plant under investigation i.e. *Acacia farnesiana*

Synonyms: Sweet acacia, Vachellia farnesiana.

Biological origin: Comprising of shrubs and small trees of *Acacia farnesiana* from the Leguminosae family.

Geographical origin: *Acacia farnesiana* is native to India, specifically in Tropical Burma, Sri Lanka, Saudi Arabia, Egypt, and Africa. In India, it is predominantly found in Maharashtra, Gujarat, Andhra Pradesh, and Karnataka.

Description: *Acacia farnesiana* is characterized as a spreading shrub typically 1.5 - 4m tall, with smooth or finely fissured grey-brown bark. The leaves have petioles 0.2 - 2 cm long, are hairy especially on top, with a circular to elongated gland, occasionally with a sugary gland apex, and lack inter jugary glands. The plant bears globule heads with 33-95 flowers, yellow or orange in color, located in 1-3 axils of leaves, with mostly 3-30mm

long hairy peduncles. The pods resemble cigars, straight to strongly curved, terete, turgid, 1.5 - 8.5 cm long, 8-17 mm wide, dark brown to blackish, glabrous, with transverse or oblique seeds separated by pith.

Chemical components: Pods contain a variety of polyphenols such as Gallic acid, Ellagic acid, m-digallic acid, Methylgallate, Kaempferol, Aromadendrin, and Naringenin. Additionally, the seeds of *Acacia farnesiana* are a rich source of amino acids including Lysine, Arginine, Glycine, and Histidine.

Medical benefits of *Acacia Farnesiana* Plant

Anti-Inflammatory/Cytotoxicity: A study revealed the presence of four new diterpenes—acasiene-B, farnesiran-A, farnesirana-B along with three known diterpenes and eight flavonoids. Some of these compounds displayed cytotoxicity to human Cancer cell lines while others exhibited moderate anti-inflammatory activity.

Vibrio cholera inhibition: Research on 32 medicinal plants demonstrated that the ethanolic extracts of *A. farnesiana* and *Artemisia ludoviciana* effectively inhibited the growth of Cholera vibrio strains. The effects on enterotoxin production and adhesion were also investigated.

Antihyperglycemic Activity: A study was conducted to assess the anti-hyperglycemic activity of an active fraction derived from an aqueous extract in Alloxan induced diabetic rats. The results demonstrated promising anti-diabetic effects, with no noticeable toxic symptoms observed in the active fraction. Another study evaluated the anti-hyperglycemic activity of *Acacia farnesiana* extracts, and it was found that a water extract significantly reduced blood glucose levels. The activity was primarily observed in the soluble fraction, suggesting a direct stimulatory effect on glucose uptake without the involvement of insulin, which could be the main mechanism.

Antiulcer/Adsorbent: The ulcer healing activity of *Acacia farnesiana* methanol leaf extract was evaluated in

a rat model of ulcer induction. The results showed a significant reduction in the ulcer index compared to the control group treated with Ranitidine, indicating the potential of the methanol extract in promoting ulcer healing.

Bronchodilator/Anti-Inflammatory: A study investigated the effects of a glycosidal fraction obtained from unripe pods of *Acacia farnesiana* on smooth muscle relaxation and inflammation. The results revealed a direct relaxant effect on bronchial muscles and inhibition of carrageen and formaldehyde-induced inflammation, indicating its potential as a bronchodilator and anti-inflammatory agent.

Antioxidant/Protection against Oxidative Induced Damage: The antioxidant properties of *Acacia* pods extracts, specifically *Acacia Schaffer* and *Acacia farnesiana*, suggest that the antioxidant components present in these pods can transfer to animal products such as milk, meal, and by-products when included in their diet. This transfer of antioxidants can provide protective effects against oxidative-induced damage.

Antibacterial/Antioxidant/Anti-Inflammatory: In a study evaluating ethanolic extracts of five plants, including *Acacia farnesiana*, *S. alata*, *S. grand flora*, *S. cumini*, and *T. divaricata*, all extracts exhibited antioxidant and antibacterial activity. Furthermore, these extracts demonstrated anti-inflammatory effects by reducing interleukin (IL)-6 secretion and/or tumor necrosis factor (TNF)- α production.

Experimental section: Further as a part of the search aimed at screening Indian plant *Acacia farnesiana* over a broad range of biological activities being carried on at the Central Drug Research Institute (CDRI), Lucknow (INDIA). It was found that 90% Ethanolic extract of *Acacia farnesiana* exhibited oxytocic activity. As a result of this work, five pure substances (compounds) were isolated and characterized.

Table I.

S. No.	Substances	Molecular formulae	m.p. ($^{\circ}$ C)	Identification
1	Substance - A	C ₃₀ H ₆₂ O	70	Triacantanol
2	Substance - B	C ₂₉ H ₅₀ O	136	β -Sitosterol
3	Substance - C	C ₃₅ H ₆₀ O ₆	288	β -Sitosterol- β -D-glucoside
4	Substance - D	C ₁₅ H ₆₀ O ₆	330	Luteolin (Tetrahydroxyl flavones)
5	Substance - E	C ₂₁ H ₂₀ O ₁₂	219	Isoquercitrin

Isolation of the Individual constituents from *Acacia farnesiana*: **Substance – A:** It was crystallized from methanol as a white micro crystalline solid, m.p. 70 $^{\circ}$ C. its analytical data showed it to have the molecular formula C₃₀H₆₂O which was confirmed by M⁺ peak at m/z 438 in the mass spectrum. Its infrared spectrum showed absorption at 3300 (OH), 2900 cm⁻¹ (-CH stretching) and other peaks in the finger print region. The

PMR spectrum displayed a triplet at 0.80 ppm integration for a methyl having an adjacent methylene, a broad multiple at 1.1 – 1.4 ppm integration for 56 protons due to methylene (28 x CH₂), and a two protons triplet at 3.57 ppm (J= 6 Hz) characteristic for carbinolic protons of a primary function (-CH₂OH) in its mass spectrum the molecular ion peak at m/z 438 showed a facile loss of H₂O molecule (m/z 420, M-18) and cluster of peaks due

to subsequent losses of methylenes. This indicated it to be straight chain alcohol.

Substance – A formed monoacetate (M^+ , 480) whose infrared spectrum showed the absence of hydroxyl group but indicate the presence of an acetyl carbonyl at 1720 and 1240 cm^{-1} . Its PMR spectrum revealed the presence of an acetoxymethyl at 2.1 ppm (3H, s) and a triplet centered at 4.15 ppm ($J = 6$ Hz) due to the $-\text{CHOAc}$ group. The mass spectrum of monoacetate did not a cluster of peaks due to subsequent loss of methylenes.

Physio-Chemical data of substance-A and its acetates were identical with those of triacantanol²¹ and its monoacetate derivatives.

Substance – B: It was crystallize from methanol as colorless needle like solid having m.p. 136°C. ($\alpha_D - 35^\circ$ (c, 1.0 CHCl_3). It showed positive Liebermann-Burchard (LB test) color reaction and formed a monoacetyl derivative, m.p. 125°C. it was identified as β -sitosterol by comparison with an authentic sample.

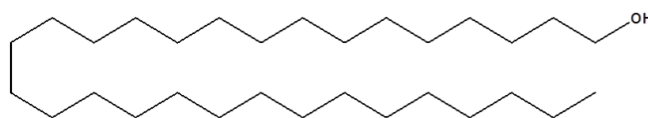
Substance – C: It was crystallized from methanol as a white micro crystalline solid, m.p. 288-290°C. It gave a positive LB-test for sterols and Feigl's test for glycoside. On acid hydrolysis it gave an aglycone, m.p. 136-137°C, which analyzed for $\text{C}_{29}\text{H}_{50}\text{O}$ and was identical of the hydrolysate showed the presence of only glucose by co-paper chromatography. The glycoside thus appeared to be β -Sitisterol- β -D-glucoside. In the mass spectrum (substance-C tetra acetate), the molecular ion peak (M^+) was not visible because the first electron impact disrupted the glycosidic linkage resulting in the formation of ion m/z 414 (β -sitosterol moiety) and ion m/z 331 (tetra acetate glucose moiety). The peak m/z lost a molecule of water to give ion m/z 396 while tetra acetate glucose moiety m/z 331, underwent stepwise fragmentation of the acetyl groups as acetic acid or ketene to yield ion m/z 229, 211, 169 (base peak) and 109 respectively.

Substance – D: It crystallize from methanol as yellow needles, m.p. 330°C and analyzed for $\text{C}_{15}\text{H}_{10}\text{O}_6$ which was confirmed by molecular ion M^+ peak at m/z 286. It gave a green colour with alcoholic ferric chloride indicating that it contain phenolic hydroxyl groups. A deep yellow colour with ammonia and magenta colour with Mg/HCl (Shinoda's Test) indicated it to be flavonoid. The IR spectrum of the compound showed bands at 3300 cm^{-1} for hydroxyl, 1670 cm^{-1} for carbonyl and 1600, 1500 cm^{-1} for aromatic ring in the molecule.

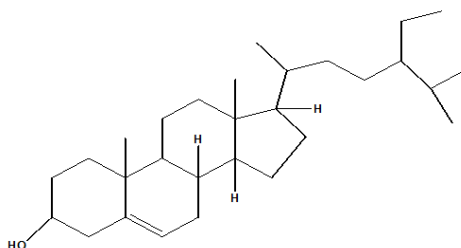
In the PMR spectrum aglycone in acetone- d_6 , it exhibited two meta-coupled protons at 6.15 and 6.40 ppm for H-6 and H-8 of the ring. It form tetra acetate, m.p. 260°C, analysed for $\text{C}_{23}\text{H}_{18}\text{O}_{10}$. Its IR spectrum was devoid of hydroxyl function. Its PMR spectrum showed the four acetoxy methyl at 2.34-2.43 ppm and other proton signals at 6.80 ppm (d, $J=2\text{Hz}$, 6H), 6.60 ppm (s, C-3H), 7.50 ppm (d, $J=8$ Hz, C-5H).

The data established the aglycone to be tetra hydroxyl flavones (Lutiolin). The physic-chemical data of aglycone was identical to that of Lutiolin and its identities was confirmed by Co-TLC and m.p.

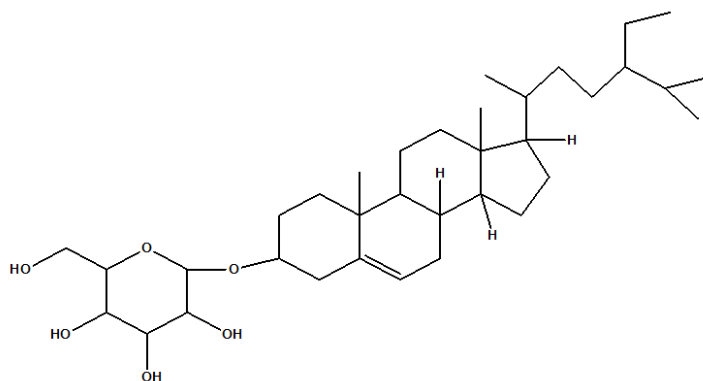
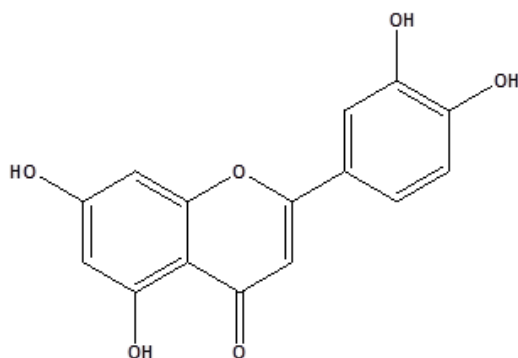
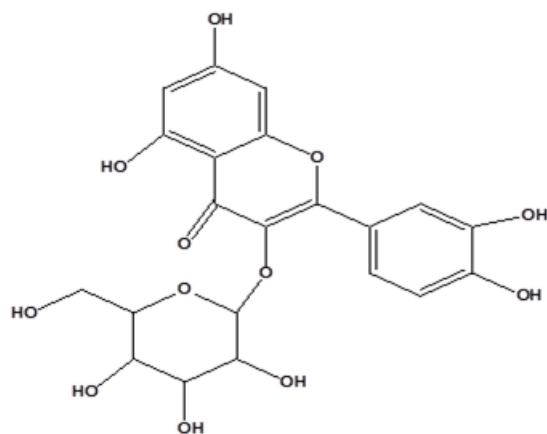
Substance – E: It crystallize from methanol as pale yellow micro crystalline solid, m.p. 230-232°C. It analyzed for $\text{C}_{21}\text{H}_{20}\text{O}_{12}$ and gave positive Feigl's test for glycoside and Shinoda's test for flavonoids. On the acid hydrolysis it yielded quercetin as aglycone and glucose as the sugar. The molar ratio of glucose quercetin was found to be 1:1 as determined by standard fehling's method. Methylation of substance E with dimethyl sulphate and anhydrous potassium carbonate in dry acetone yielded a per methyl derivative which an acid hydrolysis gave tetra methyl quercetin, which was identified through m.p. and Co-TLC with an authentic sample. The formation of tetra methyl quercetin indicated that glucose in substance E was attached to C-3 position. Substance E was, therefore confirmed as quercetin 3-O-D-glucopyranoside i.e. isoquercetin which was reported earlier by Hermann.^[22]



(A) Triacantanol



(B) β - Sitosterol

**β-Sitosterol-D-glucoside****(D) Luteolin tetra-hydroxy flavone****(E) Isoquercitrin**

Biological and anti-fertility activity of *Acacia farnesiana*: The various constituents will be isolated according to the standard procedure available in literatures. Analysis of essences of the floral extract from this plant, long used in perfumery, resulted in the name for the sesquiterpene biosynthetic chemical farnesol, found as a basic sterol precursor in plants, and the cholesterol precursor in animal. The bark is used for its tannin content. Highly tannin barks are common in general to acacias, extract of many being are used in medicine for this reason.

The bark and flowers are the part of tree most used in traditional medicine. *Acacia farnesiana* has been used in Colombia to treat malaria, and it has been confirmed in the laboratory that extract from the tree bark and leaves is effective against the malarial pathogen *Plasmodium falciparum*. Indigenous Australians have used the roots and bark of the tree to treat Diarrhea and skin diseases.

CONCLUSIONS

The bark, leaves and root of *Acacia farnesiana* are very useful for anti-fertility activity. *Acacia farnesiana* was assessed for acute oral toxicity in Wistar rats according to OECD 423 guidelines. No clinical pathogenesis or mortality was observed, indicating its safety under laboratory conditions. The all the above five compounds (A, B, C, D & E) are tested over rats and found very remarkable results towards anti-fertility activity. The percentage inhibition of acute anti-fertility activity was greater in the test group compared to the standard drug-treated animals.

ACKNOWLEDGEMENT

The authors are thankful to the Pharmaceutical division and Director (RSIC) CDRI, Lucknow, INDIA for their co-operation and valuable help for analytical and spectral analysis (recording IR and PMR spectra). The financial support received from U.G.C. New Delhi and we are also thankful to Prof. H.C. Rai, B.R. Ambedkar Bihar University Muzaffarpur, India for moral support and providing Laboratory facilities. We are also thankful to Prof. A. K. Srivastava, Department of Chemistry, B.R. Ambedkar Bihar University Muzaffarpur, India for valuable guidance, discussions and sustained encouragement.

REFERENCES

1. Saha J C, E C, Kashinathan N. Ecobolic properties of Indian medicinal plants, Part-I. Indian J. Med. Res., 1961; 49(1): 130-151.
2. Chaudhary R R. Plant with possible anti-fertility activity, special report series No. 55, ICMR, New Delhi, 1956.

3. Malhi B S, Trivedi V P, Vegetable anti-fertility drugs of India, Q.J. Crude Drugs Res., 1972; 12(30): 1922-1928.
4. Bhaduri B, Ghosh C R, A N, Moza B K, Basu B P. anti-fertility activity of some medicinal plants. Indian J. Exp. Biol., 1968; 6: 252-253.
5. Pakrashi A, Basak B Mokerjee N. search for anti-fertility agents from indigenous medicinal plants. Indian J. Med. Res., 1975; 63(3): 378-381.
6. Dhawan B N, Dubey M P, Mehrotra B N, Rastogi R P, Tandon J S. Screening for Indian plants for Biological activity. Pt.IX, Indian J. exp. Biol., 1980; 18(6): 594-606.
7. Yasnetsov V S, Sizov P I. Farmakal. Toksikol, 1972; 35: 201. Chem. Abstr., 1972; 77: 14212.
8. Aizov P I. Zdavookhr Beloruss. 1970, 16, 17. Chem. Abstr., 1970; 73: 108122.
9. Ibid, Idem. 1969, 15, 44. Chem. Abstr., 1970; 72: 119957.
10. Horikoshi S. Jap. J. Med. Sci. IV Pharmacol., 1933; 7: 103. Ibid., 1937; 10: 190.
11. Gruber C M, Brundage J T, Heiligman R. J. Pharmacol, 1935; 55: 430.
12. Garg K N, Sharma S, Bala S. Jap. J. Parmacol, 1973; 23: 195; Chem. Abstr., 1973; 79: 100416.
13. Machi B S, Trivedi V P. Quart. J. Crude Drugs Res., 1972; 12: 1922.
14. Fransworth N R, Bigel A S, Cordell G A, Crane F A, Fong H S. J. Pharm. Sci., 1975; 64: 545; Ibid, 1975; 64: 717.
15. Srivastava A K, Singh Pratima. Studies on medicinal plant (*Rubus elliptical*) with Potential anti-fertility activity. J. Chemtracks, 2017; 19(2): 283-286.
16. Srivastava A K, Singh Pratima. Studies on medicinal plant (*Juniperus communis*) with Potential anti-implantation activity. J. Chemtracks, 2017; 19(2): 295-298.
17. Ashwini M, Mujumdar AM. Antidiarrhoeal activity of methanolic extract of *Acacia farnesiana* Bark. Asian Journal of Chemistry, 2011; 23(4): 1767-70.
18. Batiha GE, Akhtar N, Alsayegh AA, Abusudah WF, Almohmadi NH, Shaheen HM, Singh TG, De Waard M. Bioactive compounds, pharmacological actions, and pharmacokinetics of genus *Acacia*. Molecules, Oct. 28, 2022; 27(21): 7340.
19. Delgadillo Puga, C., Cuchillo-Hilario, M., Navarro Ocaña, A., Medina-Campos, O.N., Nieto Camacho, A., Ramírez Apan, T., López-Tecpoyotl, Z.G., Díaz Martínez, M., Álvarez-Izazaga, M.A., Cruz Martínez, Y.R. and Sánchez-Quezada, V., Phenolic compounds in organic and aqueous extracts from *Acacia farnesiana* pods analyzed by ULPS-ESI-Q-oe/TOF-MS. In vitro antioxidant activity and anti-inflammatory response in CD-1 mice. Molecules, 2018; 23(9): 2386.
20. Gabr S, Nikles S, Wenzig EM, Ardjomand-Woelkart K, Hathout RM, El-Ahmady S, Motaal AA, Singab A, Bauer R. Characterization and optimization of phenolics extracts from *Acacia* species in relevance to their anti-inflammatory activity. Biochemical Systematics and Ecology, Jun. 1, 2018; 78: 21-30.
21. Karizone, Tatsuo, Isoi, Koichin. J. Pharm. Japan, 1976; 76: 473; Chem. Abstr., 1956; 50: 1598h.
22. Geissman T A. J. Am. Chem. Soc., 1942; 1704.
23. Clark H, wander S H. J.Am. Chem. Soc., 1953; 75: 50.
24. Hermann K. Naturwissen Cheften., 1956; 43: 158; Chem. Abstr., 1958; 52: 13898B.