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Phyto-chemical analysis and antifungal activity of aerial parts of *Acacia farnesiana (L.) Willd*

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Abstract
One of the medicinal plants that seem promising is *Acacia farnesiana (L.) Willd.* a thorny shrub found throughout Native of tropical South America, now Pan Tropical, distributed in India, Pakistan,Nepal, Sri Lanka, Maldives, Myanmar and Andaman Islands. In Bangladesh, it is naturalized and found frequently along railway tracks, in village thickets and outskirts or in waste fallow lands in most of the districts. In the hill districts, the species is found under cultivation near tribal houses. It is a well-known medicinal plant commonly used in humans as an immune system booster. Acacia farnesiana (L.) Willd.is an herbaceous plant in the family Mimosaceae, native to India and Sri Lanka. The active chemical constituents of *Acacia farnesiana (L.) Willd.* Which have been identified so far include diterpene lactones and flavonoids. The main diterpenoids that have been isolated from *Acacia farnesiana (L.) Willd.* Are aromadendrin and 14-deoxy-11, 12-didehydroaromadendrin.
The aerial parts of the plant (leaves and stems) are used to extract the active phytochemicals. The leaves contain the highest amount of aromadendrin (2.39%), the medically most active phytochemical in the plant, while the seeds contain the lowest amount.
Acacia farnesiana (L.) Willd. Has been extensive studied, most of it in the last half of the 20th century and much of it concentrating on "JG's" pharmacological composition, safety, efficacy, and mechanisms of action. The fungal culture was grown and maintained on a Potato Dextrose Agar medium. The antifungal activity of Aromadendrin solution was investigated by using the method of zone of inhibition.
Pure culture of microorganisms that form discrete colonies on solid media, e.g., yeasts, most bacteria, many other microfungi, and unicelluar microalgae, may be most commonly obtained by plating methods such as streak plate method, pour plate method and spread plate method.

Keywords: antifungal, aromadendrin, plating method

Introduction
Acacia farnesiana (L.) Willd. (Mimosaceae) is an evergreen thorny shrub, a much branched thorny shrub or small deciduous tree, up to 4 m high, bark dark brown, smooth or fissured in old trees, branchlets zigzag, lenticellate with stipular straight spines, up to 3 cm long. Leaves bipinnately compound, rachis about 2.5-7.5 cm long, pubescent, with a minute gland on the petiole, pinnae 2-8 pairs, often up to 3 cm long with a cup-shaped gland below the lowest and often at the base of the uppermost pair of pinnae, often pinnae terminated by minute bristles, leaflets 10-20 pairs, 2-7 x 0.8-1.8 mm, sessile, oblong, opposite, glabrous, base truncate, asymmetrically acute and mucronate at the apex, midrib excentric. Flowers bright yellow, very sweet-scented in axillary pedunculate, globose heads, peduncles 3.5-4.0 cm long, 3-5 together in fascicle. Fruit a pod, 3.6-7.8 x 1-2 cm, cylinderic-oblong, straight or slightly curved, subterete and turgid, dark brown to blackish when inconspicuous, indehiscent. Seeds 12-20 per pod, embedded in pulp in two rows, 7-8 x 5.5 mm, oblong-ellipsoid, smooth, black. Ayurveda is traditional system of medicine of India. It is a qualitative, holistic science of health and longevity, a philosophy and system of healing the whole body, mind of individual (Mukharji *et al*., 2005). Many herbal secondary metabolites, chemical compounds and formulations have been studied for their biological actions related to prevent human disease (Krushna *et al*., 2009).
Acacia farnesiana (L.) Willd. The Kalmegh of Ayurveda is an erect annual herb extremely bitter in taste in each and every part of the plant body. The plant is known in north-eastern India as ‘Maha-tita’, literally ‘king of bitters’ and known by various vernacular names. It is also known as ‘Bhui-neem’, since the plant, though much smaller in size, shows similar appearance and has bitter taste as that of Neem (Azadirachta indica). The genus Justicia consists of 18 species of small annual shrubs essentially distributed in tropical Asia. Only a few species are medicinal, of which *Acacia farnesiana (L.) Willd* is the most popular.

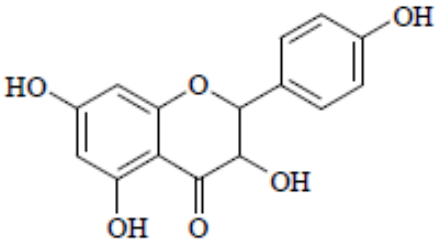
Flowering and fruiting: November to March, but in some areas throughout the year.

Distribution
Native of tropical South America, now Pan Tropical, distributed in India, Pakistan, Nepal, Sri Lanka, Maldives, Myanmar and Andaman Islands. In Bangladesh, it is naturalized and found frequently along railway tracks, in village thickets and outskirts or in waste fallow lands in most of the districts. In the hill districts, the species is found under cultivation near tribal houses.

Propagation and management: Propagation is done by seeds.

Chemical constituents

Isorhamnetin-3, 7-glucorhamnoside, gallic acid, ellagic acie, m-digallic acid, methyl gallate, kaempferol, aromadendrin, naringenin, kaempferol-7-diglucosie, naringenin-7-glucoside and a new glycoside, probably naringenin-7-diglucoside acylate with gallic acid have been isolated from flowers. Flowers also contain *d*-pinitol a pigment, isorhamnetin-3, 7-glucorhamnoside. Pods yield a new acylglucoside charcaterised as naringenin-7-O-β-D-(6″-O-galloyl) glucopyranoside (purin-O-6″-gallate); rutin and apigenin-6, 8-bis-C-glucopyranoside (0.4%). A novel amino acid N-acetyl-L-djenkolic acid has been isolated from seeds. Leaves contain tannins, alkaloids; also rutin and apigenin-6, 8-bis-C-glucide; cyanogens; linamarin, lotaustralin and an unidentified one.

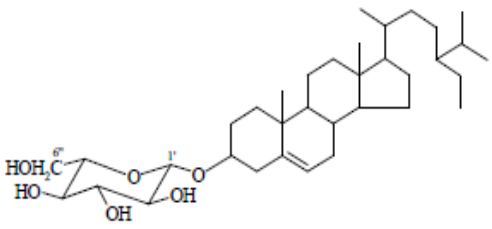


Aromadendrin

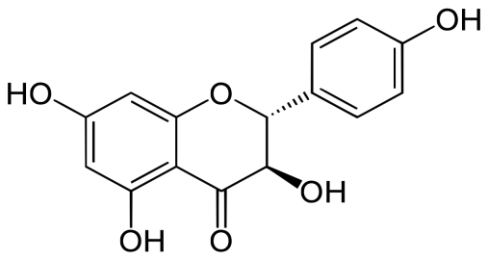
Morphology and chemistry

*Acacia farnesiana (L.) Willd.*is an annual - branched, erect - running 1/2 to 1 meter in height. The aerial parts of the plant (leaves and stems) are used to extract the active phytochemicals.

The leaves contain the highest amount of aromadendrin (2.39%), the most medicinally active phytochemical in the plant, while the seeds contain the lowest. The primary medicinal component of *Acacia farnesiana (L.) Willd* is aromadendrin. It has a very bitter taste, is a colorless crystalline in appearance, and is called a "diterpene lactone" - a chemical name that describes its ring like structure (see diagram at left). Besides the related bitters cited above, other active components include 14-deoxy-11, 12-didehydroaromadendrin, homoaromadendrin, and β-sitosterol-β-D-glycoside. Extraction is usually performed using ethanol, and liquid extracts or tinctures are the most common form of dispensing the product. When consumed, aromadendrins appear to accumulate in organs throughout the viscera.



B-sitosterol-β-D-glycoside.



Aromadendrin (C15H12O6)

Known Mechanism of action

Acacia farnesiana (L.) Willd. has been extensive studied, most of it in the last half of the 20th century, and much of it concentrating on "JG's" pharmacological composition, safety, efficacy and mechanism so faction. Many of the steps are involved in signal transduction are well understood, although research can be done to fine-tune an understanding, of these pathways. Investigating what can go wrong at such a basic level (inside the cell) allows researchers to detect diseases at a much earlier stage -- before there are obvious symptoms and when there is still a good chance to correct the problem.

"Several studies have looked at the disposition of aromadendrin in various organs of the body. Bio distribution experiements have been done in experimental animals. Following injection of radioactively labeled aromadendrin, this compound JG appears to be widely distributed in the body. High concentrations are noted in the central nervous system (brain and spinal cord) and other organs with high blood flow, including the colon, spleen, heart, lungs, and kidneys. Aromadendrin JG appears to have a relatively short half-life of JG proximately two hours. The term "half-life" refers to the time when the concentration of the compound in the body is half of what it originally was when it entered the body. This is what is left after the compound has been metabolized (broken down), changed into other forms (called metabolites), and excreted by one of several routes (urine, feces, exhaled air, sweat, or other body excretions). Compounds with short half-lives need to be given often since they do not stay in the body for long. Aromadendrins are excreted fairly rigidly from the body via the urine and gastrointestinal tract. In some studies, 80 percent of the administered dose of aromadendrin is removed from the body within eight hours, with excretion rates of more than 90 percent of the compound within forty-eight hours.

Introduction of Fungus: *Fusarium oxysporum* also referred to as panama disease or agent green, is a plant pathogenic fungus that causes ' *Fusarium* wilt' in more than a hundred species of plants such as tomato, potato, sugarcane, cowpea, *Musa spp* ., pea, ginger, etc. It colonizes the xylem of the host plant, and as a result, blockage and breakdown of the xylem leads to wilt disease symptoms such as, leaf wilting, yellowing and eventually the death of the plant. Management of *F. oxysporum* is required, as this pathogen and its many special forms affect a wide variety of hosts of economic value.

The development of resistance to common fungicides and increasing restrictions on the use of toxic material in the environment has given an impetus to the search for novel plant protectants that interfere with the fungal pathogenicity

factors. Use of natural products for the control of fungal diseases in plants is considered as an alternative to synthetic fungicides, due to their lower negative impacts on the environment. Besides being harmless and non-phyto toxic, it has been proved that plant extracts exhibit effects on germination and on the viability of fungal spores as well. Several higher plants and their constituents have been successful in plant disease control and have proved to be harmless and non phyto toxic, unlike chemical fungicides.

Objective

The objective of this experiment was to analyses the quality of the sample of AF leaves to assure that they contained sufficient active compounds and isolate Aromadendrin.

Materials and methods

The phyto-chemical compounds of this powder were analyzed by colour test (preliminary test) and thin layer chromatography (confirmatory test).

Extraction of AF Leaves by successive extraction method:

Soxhlet continuous extraction JG apparatus: In this JG apparatus extraction is boiling solvent followed by percolation finally evaporation yields the extract and recovered solvent ready for the next sample.

Extraction of *Acacia farnesiana (L.) Willd.* Using solvent-

Hexane: Take 77.505gm dry Acacia farnesiana (L.) Willd. and extracted to 8.30 hrs. The extracted yield is 0.47gm.

DCM: Take 77.00gm dry *Acacia farnesiana (L.) Willd.* After hexane extraction extracted by DCM (dichloro methyl) for 5hrs, and extracted yield is 1.211gm.

MeoH: In last extraction done by Methanol. In this from 75.01gm of *Acacia farnesiana (L.) Willd.* Extracted for 4.30 hrs. Extract yield is1.318gm.

Preliminary test

Test for Alkaloids: Weigh about 0.2 gm of plant extract in separate test tube and warmed with 2% Sulphuric acid for 2 minutes. And it was filtered in separate test tube and few drops of Dragendroffs reagent were added and observed for the presence of orange red precipitates for the presence of alkaloids.

Keller-Killani Test: Weigh about 0.5 gm of plant extract in a separate test tube with 2 ml of glacial acetic acid containing a drop of ferric chloride solution. This was under layered with 1 ml of concentrated tetra oxo sulphate (VI) acid. And observe for brown ring formation at the interface (Finar, 1983).

Test for Terpenoids: Weigh about 0.5 g plant extract in separate test tubes with 2 ml of chloroform. And add concentrated Sulphuric acid carefully to form a layer. And observe for presence of reddish brown color interface to show positive results for the presence of terpenoids.

Test for reducing sugars: Take a test tube and add 2 ml of crude plant extract and add 5 ml of Distill water and filter. The filtrate was boiled with 3-4 drops of fehling solution A and B for 2 minutes. Observe for orange red precipitate which indicates the presence of reducing sugars.

Test for Saponins: Weigh about 0.2 gm of plant extract in the test tube and add 5 ml of distilled water and then heat to boil. Observe for the occurrence of frothing (appearance of creamy mass of small bubbles) which then indicates the presence of Saponin.

Test for Tannin: To small quantity of plant extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. And observe for dark green solutions that indicate the presence of tannin.

Test for Carbonyl: Take 2 ml of plant extract in separate test tubes and add few drops 2,4, di nitro phenyl hydrazine solution and shake. And observe for the presence of yellow crystals immediately for the presence of an aldehyde.

Test for Flavonoids: Weigh about 0.2 gm plant extract in separate test tubes and dissolved diluted Sodium hydroxide and add diluted Hydrochloride. And observe for yellow solutions that turn colorless. This indicates the presence of flavonoids

Test for Phlobatanin: Weigh about 0.5 gm of plant extract in a test tube and dissolve with distilled water and filter. The filtrate was boiled with 2% Hydrochloric acid solution. Observe for a red precipitate that shows the presence of Phlobatanin.

Test for Steroids: To the plant extract add 2 ml of acetic anhydride and add 0.5 gm of ethanolic extract of each sample with 2 ml of Sulphuric acid. Observe for the color change from violet to blue or green in samples indicating the presence of steriods.

Table of chemical compound

S. No	Compounds	Extract of JG leaves
1	Diterpenoids	Present
2	Flavoinds	Absent
3	Glycosides	Present
4	Reducing sugars	Absent
5	Saponins	Absent
6	Steroids	Absent
7	Tannins	Absent
8	Terpenoids	Present

Confirmatory test

Thin layer chromatography analysis was done after the colour test to confirm the AF identity.1 g of powdered AF leaves was boiled with 20 ml of ethanol in a water-bath for 5 minutes, and then 300 mg of decolorizing charcoal were added, stirred and filtered. The filtrate was evaporated under reduced pressure until dryness and the residue dissolved in 1 ml of warm ethanol (80%). As standard 2 mg of aromadendrin, 2 mg of neo-aromadendrin and 4 mg of dehydro-aromadendrin were dissolved each in 1 ml of ethanol by using adsorbent silica gel GF254 and mobile phase chloroform (absolute ethanol 85:15). 5 microliter was used for 40each spot. Migration path was 15 cm (ascending). After quenching by UV radiation (lambda=254 nm) detection was made by spraying with 2% w/v 3, 5-dinitrobenzoic acid excess of 5.7% w/v potassium hydroxide.

Work on Hexane part

The hexane fraction was chromatographed over silica gel (100gm) eluting with hexane, and elutents increasing polarities using varying proportion of EtOAc and MeOH to

provide 1-2.

The Column chromatography was performed on silica gel and spots were visualized by exposure to I_2 vaours and or 10% sulphuric acid sprays, followed by heating at 110^0C. Details of fractions of hexane extract are given in Table 1.

Table 1: Details of fractions of hexane extract.

S.No	Eluent	Fraction no.	No of Fraction collected
1	Hexane	1-10	10
2	EtOAc:Hexane (5:95)	11-15	5
3	EtOAc:Hexane (10:90)	16-25	9
4	EtOAc:Hexane (15:85)	26-40	16
5	EtoAc:Heaxane (25:75)	57-62	5
6	EtOAc:Hexane (1:1)	91-102	11
7	EtOAc:Hexane (75:25)	116-125	10
8	EtOAc	141-150	9
9	EtOAc:MeOH (5:95)	151-160	9
10	EtOAc:MeOH (25:75)	161-165	9
11	MeOH	166	Column washed

Table 2: For pooled fraction

Name of Pooled Fraction	Fraction no.	Developing solvent system for TLC
A (Trans-phytal)	1-15	Hexane
B	16-33	$CHCl_3$:Hexane(10:90)
C	34-60	$CHCl_3$:Hexane)(10:90)
D	61-90	$CHCl_3$:Hexane(1:1)
E	91-151	$CHCl_3$:Hexane(75:25)
F(14-deoxyaromadendrin)	152-158	MeOH:$CHCl_3$ (10:90)

Fraction A was re-chromatographed and provided a viscous mass compound AF-1,[α] D +0.98 ^{0}C Rf 0.56($CHCl_3$:hexane10:90).By comparison of its physical and spectral data with their literature, it was confirmed as trans-phytol.

Fraction F was further chromatographed 14-deoxyaromadendrin and provided a solid compound AF-2, mp.171^{0}C [α] D, -28.5^0. RF 0.50 (MeOH: EtOAc 3:97), crystallized from Methanol. By comparison of its physical and spectral data with their literature, it was confirmed as14-deoxyaromadendrin.

Work on EtOAc part

The EtOAc fraction was chromatographed over silica gel eluting with hexane, and eluents increasing polarities using varying proportion of EtOAc and MeOH to provide compound 3.

The column chromatography was performed on silica gel (60-120 mesh) and the spots were visualized by exposure to I_2 vapour and or 10% sulphuric acid spray, followed by heating at 110^0C. Details of column chromatography of ethyl acetate are following.

Table 3: Column chromatography of ethyl acetate

S.No.	Eluant	Fraction No.	No of column washed
1	EtOAc:Hexane(1:1)	1-10	10
2	EtOAC	11-20	10
3	MeOH:EtOAc(5:95)	21-30	10
4	MeOH:EtOAc(25:75)	31-40	10
5	MeOH:EtOAc(1:1)	41-50	10
6	MeOH	51	Column washed

Table 4: Details of pooled fractions

Name of pooled fraction	Fraction no.	Developing solvent system for TLC
A	1-15	$CHCl_3$:Hexane(75:25)
B	16-20	MeOH:$CHCl_3$ (15:85)
C (Aromadendrin)	21-31	MeOH:$CHCl_3$ (25:75)
D	32-40	MeOH:$CHCl_3$ (35:65)

Fraction C was chromatographed and yielded a compound AF-3, mp.172-221^{0}C[α] D,-92^{o}C, RF 0.46(MeOH: $CHCl_3$, 5:95), crystallized from methanol. By comparison of its physical and spectral data with their literature data, it was confirmed as aromadendrin.

Method to identify the active constituents (diterpene lactones) of AF powder.

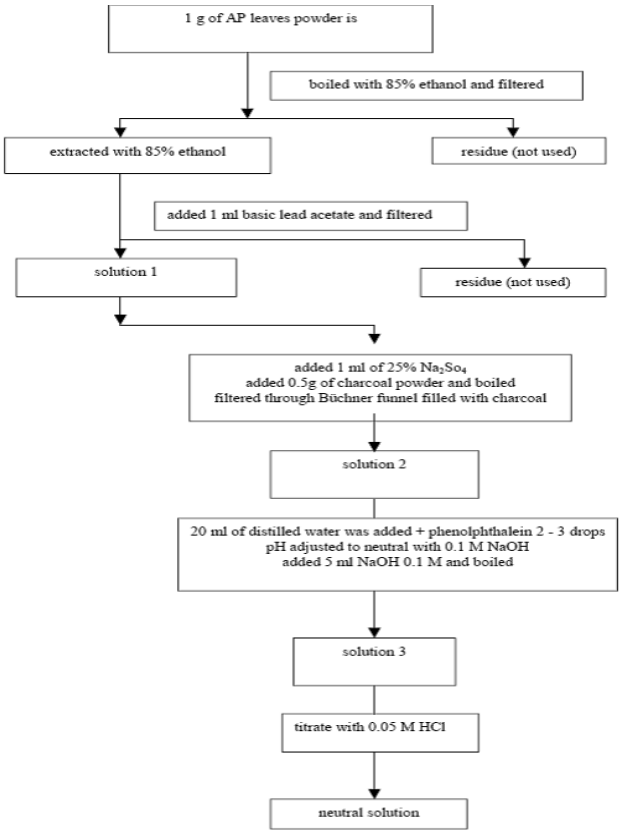


Fig 1: Antifungal activity

The plant pathogenic microorganism *F. oxysporum* (MTCC 7678) was procured from the Microbial Type Culture Collection (MTCC), in Chandigarh. The fungal culture was grown and maintained on a Potato Dextrose Agar medium. The antifungal activity of Aromadendrin solution was investigated by using the method of zone of inhibition.

Media Preparation

Steps involved in the preparation of PDA medium are –

- 20 ml of sterilized distilled water is taken into a clean conical flask.
- 40 gm of potato infusion is added.
- 4 gm of dextrose is added.

- Into this 3 gm of agar in added.
- The solution is well mixed and the solution is made upto a volume of 200 ml by adding distilled water.
- The solution is heated on a hot plate for the proper dissolution.
- The medium is sterilized by autoclaving at 15 psi pressure, 121^0c temperature for 15 minutes.

Common Methods of isolation of pure culture

Pure culture of microorganisms that form discrete colonies on solid media, e.g., yeasts, most bacteria, many other micro fungi, and unicellular microalgae, may be most commonly obtained by plating methods such as streak plate method, pour plate method and spread plate method.

Inoculation Procedure

The steps involved in the inoculation procedure areas;

1. The tube containing inoculums and the tube containing agar slant are held in the left hand and the inoculation loop/needle in the right hand. Tubes should almost be parallel to the ground to avoid contamination.
2. Both the tubes are opened by removing the cotton-plug with fingers of the right hand and the open mouth of the tubes is sterilized by passing through the flame twice.
3. Immediately after de-plugging and sterilizing the mouth of the tubes, the loop/needle is also flame-sterilized and is inserted within the agar surface of the inoculums containing tube to quench the heat, and a small bit of inoculums is taken on the loop/needle tip.
4. The inoculums containing loop/needle is taken out and brought in within the agar slant containing tube where the inoculums are just rubbed on the surface of the agar slant.
5. As the steps starting from plug removal from the mouth of the tubes to the rubbing of the inoculum on the surface of the agar slant should be taken quickly to avoid contamination.
6. When inoculation is complete, the open mouths of tubes and the cotton plugs are sterilized by flame and the cotton plugs are replaced.
7. The inoculated tube is incubated under suitable temperature to favour rigid growth of microorganisms.

Antagonistic activity of *Aromadendrin* against A. nigar

After the inoculation procedure of Fungus prepared solution/dilution of aromadendrin (1.5mg in 100µl) is taken and in petri plate dipped 4 disc of filter paper. After them marked as one control, On 2nd AF applied 10µl, on 3rd AF applied 20µl and on 4th filter disc applied 40µl and incubated for 48hrs.

After 48 hrs saw zone of inhibition and measured them.

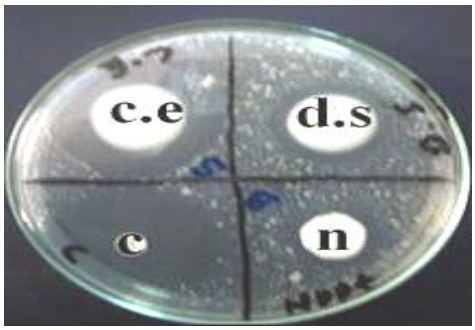


Fig 2: Zone of inhibition of A. Niger culture by different aromadendrin dilution

C: without aromadendrin dilution.
N: filter paper disk dipped in 10µl Aromadendrin dilution.
d.s: filter paper disk dipped in 20µl Aromadendrin dilution.
Ce: filter paper disk dipped in 40µl Aromadendrin dilution.

Antagonistic activity of *Aromadendrin* against fusarium

Same procedure follows against fusarium.

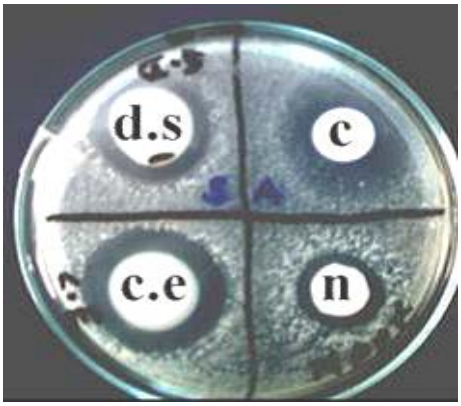


Fig 3: Zone of inhibition of Fusarium by different Aromadendrin dilution

C: without aromadendrin dilution.
N: filter paper disk dipped in 10µl Aromadendrin dilution.
d.s: filter paper disk dipped in 20µl Aromadendrin dilution.
Ce: filter paper disk dipped in 40µl Aromadendrin dilution.

1. Result and Discussion

Results of confirmatory test

The result of the thin layer chromatography are given in Table 5

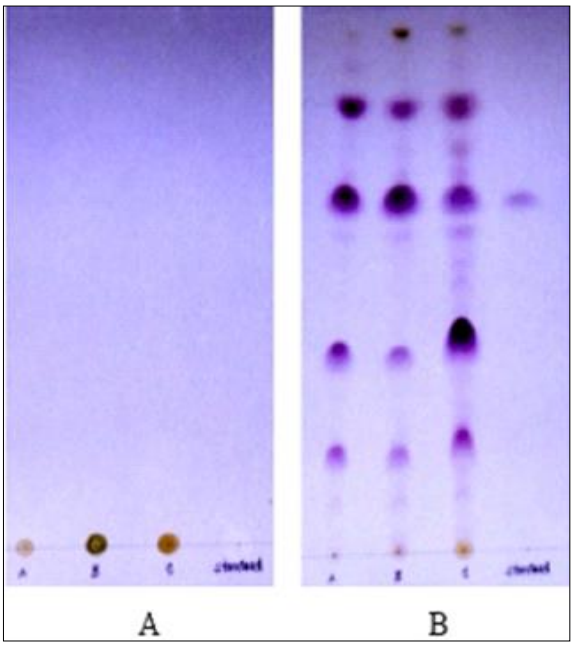


Fig 4: Thin layer chromatography of AF leaves powder before placed in chromatographic tank (A) and after development (B) a - c: AF solvent sample, standard: aromadendrin.

Table 5: Thin layer chromatography- confirmatory test

Diterpene lactones	Rf values	Development(UV254)
Lactone type 1	1-5	-
Lactone type 2	11-15	opacity
Lactone type 3	18-22	opacity
Neo-aromadendrin	28-32	-
Lactone type 5	49-51	-
Aromadendrin	52-56	Opacity
Lactone type7	57-59	Opacity
Lactone type 8	66-68	-
Dehydro-aromadendrin	69-71	opacity

Results of chemical content of AF

The following results were obtained: foreign matter 0%, water 8.58%, acid-insoluble ash 1.39%, 85% ethanol extractives 18.10% and active constituents (total diterpene lactones calculated in aromadendrin) 7.30%.

Table 6.2: Chemical content of JG

Parameter	AF leaves sample	Standard (%)
Ash value	13.5	<20.0
Acid insoluble Ash	1.08	<5.1
Foreign organic matter	0.01	<2.0
Alcohol soluble extractive	24.58	>24.00
Water soluble extractive	21.63	>20.00

Discussion

The primary tests which only show the presence of compounds which reacts chemically like the active compounds of AF. To prove that the sample contain AF further tests in the laboratory are necessary. The thin layer chromatographic test showed that the AF plant sample had the same RF values (line spots and colour) as the diterpene lactones standards which were used for comparison in this test. So it can be stated that the plant sample contains the three active compounds of AF plants: dehydro-aromadendrin, aromadendrin and neo-aromadendrin. The TLC method is used to confirm the result from the colour test by the same offices which do the colour test.

Result of Isolation of Aromadendrin

The EtOAc fraction was chromatographed over silica gel eluting with hexane, and eluants increasing polarities using varying proportion of EtOAc and Methanol to provide compound AF-1 to AF-3. The column chromatography was performed on silica gel (60-120mesh) and spots were visualized by exposure to I_2vJGours and or 10% sulphuric acid sprays, followed by heating at 110^0C.

Table 6.3: Isolate of *Acacia farnesiana (L.) Willd.*

S.No	Compounds	Extract	M.F	M.P (^{0}C)	Identified as
1	AF-1	Ethyl acetate extract	$C_{20}H_{40}O$	Viscous	Trans-phytol
2	AF-2	Ethyl acetate extract	$C_{24}H_{34}O_4$	172-173	14-deoxy andro grapholide
3	AF-3	Ethyl acetate extract	$C_{20}H_{30}O_5$	220-221	Aromadendrin

Compound AF-1: β-sitosterol-β-D-glycoside

Fraction from 5% EtOAc: Hexane solvent system, compound AF-1 was obtained. [α] D,+0.98.It was homogenous on TLC plate in different solvent systems, Rf 0.43(ethyl acetate:hexane,10:90).IR spectrum of compound AF-1 showed the presence of hydroxyl group at 3300(O-H),1670(C=C) 790cm^{-1}.^{1}H-NMR($CDCl_3$) of compound AF-1 showed(3H,s ,>C=CH3) at δ 1.65 and(2H,d, J=7Hz,=C-CH2-O-) at δ 4.12. ^{13}C-NMR of the compound AF-1 showed the signals at 123.56 and 138.12 for C-2 and C-3 respectively.

A molecular ion peak of the compound AF-1 was observed in the mass spectrum at m/z 296 which corresponded to the molecular formula $C_{20}H_{40}O$. The other ion fragmentations at m/z 278,262,248.235,222,209,237,121,98;85,81,71,57,43. On the comparison of its physical and spectral data with those reported in the literature, it is confirmed as β-sitosterol-β-D-glycoside.

Compound AF-2 (Aromadendrin)

Fraction from 5% MeOH:EtOAc solvent system, a colourless solid was obtained, crystallized from methanol ,mp.172-221^{0}C, ,[α] D ^{0}C -92 ^{0}C.It was homogeneous on TLC plate in different solvent system, Rf 0.46(methanol: ethyl acetate,5:95).IR spectrum of compound showed the presence of hydroxyl gp at 3340-3200,α ,β –unsaturated γ-lactone portion at 1725, double bond at 1667 cm$^{-1.}$ ^{1}H-NMR (CDCl3) spectrum of compound JG-3 showed the methyl gp at 0.86 and 1.07 and the exo methylene gp at δ 4.58 and δ 4.77.

A molecular ion peak of the compound AF-3 was observed in the mass spectrum at m/z 350 which corresponded to the molecular formula $C_{20}H_{30}O_5$.On the comparison of its physical and spectral data with those reported in the literature, it is confirmed as aromadendrin.

Compound AF-3 (Aromadendrin)

Fraction from 1% MeOH: EtOAc solvent system, a colourless solid was obtained, crystallized from MeOH, mp.172-173^{0}C, [α] D-28.5^{0}C. It was homogenous on TLC plate in different solvent systems, RF 0.50(methanol: ethyl acetate, 3:97). IR spectrum of compound AF-1 showed the presence of hydroxyl group at 3630,α ,β –unsaturated γ-lactone portion at 1755,double bond at 1640cm^{-1}.^{1}H-NMR($CDCl_3$) spectrum of compound AF-2 showed the methyl group at δ 0.62 and δ 1.22 and exo methylene group at δ 4.55 δ 4.84 and δ4.84.

A molecular ion peak the compound AF-2 was observed in the mass spectrum at m/z 334 which corresponded to the molecular formula $C_{24}H_{34}O_4$. On the comparison of its physical and spectral data with those reported in the literature, it is confirmed as 14-deoxy aromadendrin.

Result of Antifungal activity of Aromadendrin

Table 6: Inhibition zone

S. No	Fungus	Inhibition zone diameter(mm)[a,b]		
		10µl(A.S)	20µl(A.S)	40µl(A.S)
1	A. niger	24±0.5	22±0.4	16±0.5
2	F. oxysporum	35±0.5	30±0.5	25±0.5

A: mean value± SD (the zone of inhibition (in mm) including disc of 8mm in diameter)

B: statistical analysis data are expressed as means± SD

From the table it is clear that Aromadendrin is active against Aspergilus and Fusarium. We can also see that as we increasing concentration zone of inhibition increasing. Now we can conclude Aromadendrin show antagonistic property against fungus and it shows much antagonistic peoperty against Fusarium comparison Aspergilus.

Conclusion

From Table 1 confirmatory test it is clear that leaf extract of *Acacia farnesiana (L.) Willd.* showed spots of different Rf values between 28-32, 52-56, 69-71. It is well known that Aromadendrin gives RF value b/n 52-56.

So from confirmatory test it is clear that extract of *Acacia farnesiana (L.) Willd* contain Aromadendrin.

From Table 3 it is concluded that compound JG-3 obtained from column chromatography is Aromadendrin.

It is well clear from Mass and NMR that: Fraction from 5% MeOH:EtOAc solvent system, a colourless solid was obtained, crystallized from methanol ,mp.172-221^{0}C, ,[α] D ^{0}C -92 ^{0}C.It was homogeneous on TLC plate in different solvent system, Rf 0.46(methanol: ethyl acetate,5:95).IR spectrum of compound showed the presence of hydroxyl gp at 3340-3200,α ,β –unsaturated γ-lactone portion at 1725, double bond at 1667 cm$^{-1.}$ ^{1}H-NMR (CDCl3) spectrum of compound JG-3 showed the methyl gp at 0.86 and 1.07 and the exo methylene gp at δ 4.58 and δ 4.77.

A molecular ion peak of the compound AF-3 was observed in the mass spectrum at m/z 350 which corresponded to the molecular formula $C_{20}H_{30}O_5$.On the comparison of its physical and spectral data with those reported in the literature, it is confirmed as aromadendrin. So it is fully proved that compound AF-3 is Aromadendrin. For antagonistic activity it clear that from the table 4 it is clear that Aromadendrin is active against Aspergilus and Fusarium. We can also see that as we increasing concentration zone of inhibition increasing. Aromadendrin is most active against fusarium. Now we can conclude Aromadendrin show antagonistic property against fungus and it show much antagonistic property against Fusarium comparison Aspergilus.

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