Chemical examination and antibacterial activity of heartwood of Acacia raddiana

Ranveer Singh

.Department of Chemistry, MLB Govt College, Nokha (Bikaner)

Abstract- Acacia raddiana belongs to family leguminosae. It is a medicinal plant, which has been investigated phytochemically only for polyphenols. Air dried chipped heartwood weighing (5kg) was refluxed with petroleum ether (60-80°c) over a steam bath. The defatted heartwood was then exhaustively extracted with chloroform. Extracts were mixed and fractionated with pet. ether, benzene, ethyl acetate and methanol. Column chromatography of these fractions over silica gel (60-120 mesh) afforded n-Octacosanol,3-Acetyl-β-Sitosterol, γ-Sitosterol, Betulin, Friedelin. Characterizations of these compounds were established mainly by UV and 1HNMR spectroscopy. The antibacterial activity of the heartwood extract of Acacia raddiana has been evaluated by Agar- well diffusion method. The result obtained show that heart wood extract and their fractions serve as effective agent against selected bacterias. Antibacterial efficiency dependent upon the nature of fraction and vary with respect to specific bacteria.

Key words: leguminosae, heartwood, β-sitosterol, betulin, antibacterial activity, gentamycin, agar-well diffusion.

I.Introduction

Plants play a very important role in modern medicinal sciences[1], in the introduction of new therapeutic agents[2,3]. The several plant extracts have marked anti-inflammatory[4,5], antihepatotoxic, analgesic[6], spasmolytic[7], antimicrobial[8], antibacterial9, antitumor[10], antioxidant[11,12], antifertility[13] activity and also used as anti-HIV agents[14]. A wide variety of pharmacoactive substances have been investigated from the plants that had activity efficiency[15-19].

Several plant extracts have wide application as antibacterial medicines which rarely have severe side effect[20]. Several species of Acacia are also known for antimicrobial, antifungal, anticancer and antioxidant activities[21-28]. Acetone extracts of Acacia Senegal and Acacia dealbata were phytochemically rich of phenols and flavonoids and exhibited antioxidant activities [29,30]. Acacia rigidula's root and stem extract have been used as reducing and capping agent to produce silver nanoparticles that eradicate pathogenic resistant bacteria in vivo[31]. Acacia raddiana is a medicinal plant[32], which has been investigated phytochemically only for polyphenols. Plants containing phenolic compounds are possible source of natural antioxidents that stabilize free radicals by hydrogenation or complexing with oxidizing species [33]. Various extract of this plant showed muscle relaxing activity[34] while its seed

extract exhibited antihyperglycemic activity[35]. Bacterial infection is a serious problem for our health system. Now, there is a public criticism of synthesized chemicals that are used as safe and strong antibacterial agent. Bacteria belonging to the genus Bradyrhizobium are capable of establishing symbiotic relationships with a broad range of plants belonging to the three subfamilies of the family Leguminosae (Fabaceae), with the formation of specialized structures on the roots called nodules, where fixation of atmospheric nitrogen takes place[36]. In the present phytochemically investigate the heartwood of acacia raddiana and the antibacterial activity have been evaluated for its ethanolic extract. Test bacteria like Gram +ve : Bacillus subtilis, Staphylococcus aureus and Gram –ve : Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa and Raultella planticola were screened by agar-well diffusion method[37] using gentomycin as standard.

II .*Experimental Protocol*

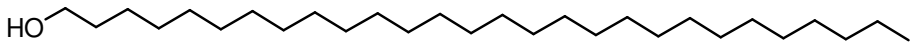
2.1. Chemical Examination-

Air dried chipped heartwood weighing (5kg) was refluxed with petroleum ether (60-80°c) over a steam bath. The defatted heartwood was then exhaustively extracted with chloroform. Removal of solvent on a boiling water bath yields a dark brownish semisolid mass. . Extracts were mixed and fractionated with pet. ether, benzene, ethyl acetate and methanol This mass was column chromatographed over silicagel and collected several fractions. These fractions were further separated by preparative TLC in order to get pure compounds.

The isolated compounds are as follows:

Compound A (n-Octacosanol)

It was isolated as colourless granules, m.p. 83-84º. Elemental analysis and mass spectrometric studies indicated its molecular formula as C28H58O.It was soluble in benzene, ethyl acetate, chloroform and acetone on warming. It developed no colour with TNM and showed no absorption in UV and visible region.



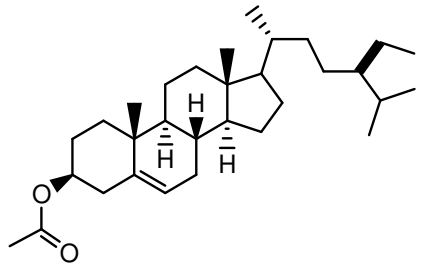
IR : ν_{max}^{KBr} : 3360, 2960, 2899, 2840, 1460, 1062, 732 and 725 cm^{-1}

Analysis : Found : C, 81.89; H, 13.82%

Calcd. for C28H58O C, 81.95; H, 14.14%

Compound: B (3-Acetyl-β-Sitosterol)

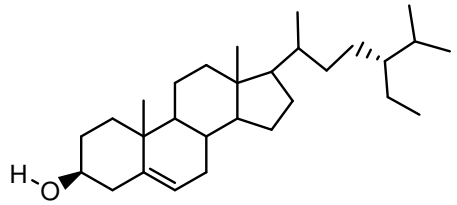
It was isolated as light yellow solid, m.p. 126º. It gave positive Liebermann - Burchad test. This test showed it to be a sterol.



IR : ν_{max}^{KBr} : 2900 (O–H str.), 1724 (>C=O) 1468, 1375 (C–H str. of –CH3 and >CH2), 1260-1250 (C–O–C bending), 1136, 1037 (C–OAc bending) 957 and 798 cm^{-1}.

Compound C (γ- Sitosterol)

It was isolated as colourless flakes, M.P. 145ºC. It gave positive liebermann Burchard test for sterol.



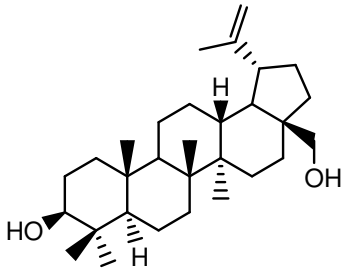
IR : ν_{max}^{KBr} : 3400, 1130, 1062, 1050, 1020, 970, 960. 850 and 735 cm^{-1}

Analysis : Found : C, 83.84; H, 12.00%

Calcd. for C29H50O C, 84.05; H, 12.08%

Compound D (Betulin)

It was isolated as white needles. m.p. 249-50°C. It gave yellow colour with tetranitromethane positive colour reaction of triterpenes viz. Liebermann-Burehard and Noller's reaction.



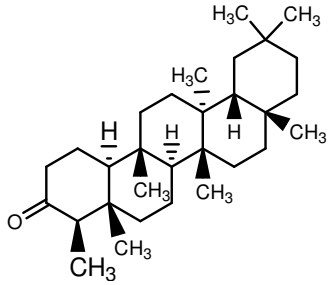
IR : ν_{max}^{KBr} : 3480-3380 (–OH str.), 2975, 2880, 1650 (C=C str.), 1380, 1370 (>C(CH3)2 bending) cm^{-1}.

Analysis : Found : C, 81.15; H, 11.15%

Calculated for C30H50O2 C, 81.45; H, 11.32%

Compound E (Friedelin)

It was isolated as colourless, needles, m.p. 258-60°C. It was soluble in benzene, ethyl acetate to TNM test. It gave colour reactions characteristic of triterpenes viz. Liebermann-Burehard test and Noller's test.



IR : ν_{max}^{KBr} : 2950, 2890, 1725, 1470, 1400, 1380, 1360, 1310, 1290, 1230, 1200, 1190, 1118, 1080, 1055, 990, 920, 850 and 795 cm^{-1}.

Ms : m/e : 426(m+), 356, 341, 302, 273 etc.

Calculated for C30H50O C, 84.45; H, 11.75%

2.2. ANTIBACTERIAL ACTIVITY-

Preparation of test extracts

For antimicrobial activity, powdered heartwood of Acacia raddiana Willd., were extracted with ethanol. The ethanolic extract was concentrated in vacuo, fractionated with pet. ether, benzene, ethyl acetate and the residue was re-extracted (2 x 8 hr) for complete exhaustion. Further the extracts/fractions were pooled individually and dried in vacuo.

All the extracts were stored at 4°C in a refrigerator until screened for a particular activity. However, their final concentration was prepared in the respective solvents, before use.

Source of test organisms

Pure cultures of test bacteria, namely Bacilllus subtilis, Enterobacter aerogenes, Escherichia coli, Pseudomonas aeruginosa, Raoultella planticola and Staphylococcus aureus, were obtained from S.M.S. lab. Jaipur (Rajasthan). These cultures were grown and maintained on Nutrient Broth Medium (NBM) at 27°C for 48 hr.

Cultures of test microbes

Stock cultures were maintained at 4°C on slops of nutrient agar. Active cultures for experiments were prepared by transferring an inoculating loop of cultures from the stock cultures to test tubes of NB Medium which were incubated without agitation for 24 hr at 37°C.

For antibacterial activity Agar- well diffusion method was adopted, because of its reproductivity and precision. The plates were prepared by pouring 20 ml of molten media into sterile Petri plates. The plates were allowed to solidify for 8 min. After that, 30 µl suspension was spread uniformly with the help of a sterile glass spreader and dried for 5 min. The wells (6 mm diameter) were punched in the plates using a sterile stainless steel borer. The test extract and control (gentamycin) was loaded in 6 mm well and the test sample was allowed to diffuse for 30 min. The plates were kept for incubation at 37°C for 24 hr. At the end of incubation, inhibition zones formed around the well were measured with a transparent scale in millimeters. The experiments were performed in triplicate and the mean value of the diameter of inhibition zones with ± standard deviation were calculated[38,39].

III. Result and Discussion

3.1 Chemical Examination-

By applying chromatographic and TLC techniques on the plant extract following compounds were isolated in pure form and characterized as-

1.	Compound A	m.p. 83º-84º	(n-Octacosanol)
2.	Compound B	m.p.126º	(3-Acetyl-β-Sitosterol)
3.	Compound C	m.p.176-90º	(γ- Sitosterol)
4.	Compound D	m.p.249-50º	(Betulin)
5.	Compound E	m.p.260-62º	(Friedelin)

All these compounds are known compounds.

3.2.Antibacterial activity-

For antibacterial assay, E. coli, which causes frequent urinary tract infections, is the most common organism used by the Roia et al.73. B. subtilis, E. aerogenes, E. coli, P. aeruginosa, R. planticola and S. aureus were chosen as the test organisms because of their high resistance to the antibacterial agents. Thus, any plant which would exhibit pronounced activity against these organism might yield an important antibiotic.

The results of antibacterial activities have been presented in Table 1. All the test extracts were found to be moderate and most active against mostly test bacterias. The heartwood of Acacia raddiana Willd. Showed significant activity against test bacteria. It was further observed that the activity was more pronounced in benzene fraction specially for E.coli. Other fractions are less effective against remaining test bacteria. Its ethanolic fraction exhibited lowest efficiency against R.planticola.

Table 1. Antibacterial activity of the heartwood of Acacia Raddiana.

Plant species	Type of extract/ fraction	Dose (mg/ disc)	Test Microbes											
			B. subtilis		S. aureus		E. coli		P. aeroginosa		E. aerogenes		R. planticola	
			IZ+	AI*	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI
Acacia Raddiana (heartwood)	EtOH	4	6.50	0.29	8.30	0.31	8.98	0.47	8.70	0.44	6.00	0.42	-	-
	Pet. ether	4	7.50	0.32	9.40	0.44	9.98	0.52	10.7	0.52	7.13	0.50	6.50	0.31
	C6H6	4	7.40	0.34	10.50	0.49	12.7	0.67	10.0	0.51	9.22	0.65	7.50	0.37
	EtOAc	4	7.20	0.31	10.20	0.48	11.5	0.60	10.0	0.51	9.00	0.64	6.30	0.30

IZ+ = Inhibition zone (in mm) including the diameter of disc (6 mm);

AI* = Activity index = Inhibition zone of sample/Inhibition zone of standard;

Standard : Gentamycin; (-) = No activity.

IV. Conclusion:

Acacia raddiana is well known for phenolic compounds and for biological activities. This time we isolate five known compounds by chemical examination. These compounds separated by column chromatography and identified by spectral datas. These compounds specified by specific test and by melting point. Heartwood extract and its fraction show antibacterial activity against all selected test bacterias exept R.plenticola. Benzene fraction of the extract show strong antibacterial activity against E.coli .

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