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ANTIBACTERIALS FROM BOERHAAVIA DIFFUSA

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ABSTRACT : *The chloroform and alcohol extracts of e plats were screened against six bacteria viz staphylococcus aureus, Escherichia Coli, proteus mirablis, salmonella typhimurium, Pseudomonas aeruginosa and Klebsiella a aerogenes. Benzene-ethyl acetate (4:1) eluate of chloroform extract sowed activity against E.Coli, S.typhimurium and P.aeruginosa. The n-butanol extract of alcohol extract was active against P. mirablis and S. typhimurium. A phenolic compound isolated fro the above fraction exhibited activity against P. mirablis only.*

INTRODUCTION:

Boerhaavia diffusa Linn. (Nyctanginaceae) Known as Punarnava in ayurveda is used as a diuretic and anti-convulsentexpectorant, analgesic, purgative, anthelmintic and febrifuge[1]. The leaves are reported to improve the yield of milk in cows[2]. The Nigerian variety of the plant sowed antibacterial activity against few bacteria. The plant showed antiinflammatory[4,5,] cardiotonic[4] and diuretic[6] activities. This paper deals with the systematic stud of the chloroform ad ethanol extracts of the roots of this plant and their various chromatographed fractions on different bacteria and to isolate the active principle.

MATERIALS AND METHODS:

The root of B.diffusa was purchased from local market, Madras It was identified and voucher specimen was deposited in the botany department of the institute. The root (2kg) was dried in shade and coarsely powdered. It was extracted successively with chloroform ad ethanol by cold percolation method in an aspirator bottle (48hrs). The solvent from the extracts were distilled on a water bat and last traces removed in vacuo.

The chloroform extract of the plant was chromatographed over silica gel (mesh 100-200) and each fraction was again reprocessed. The ethanol extract of the plant was partitioned into ethylacetate and later in n-butanol successively. The ethyl acetate and n-butanol extractive were subjected to column chromatograph over silica gel separately. The fractions were further chromatographed to isolate the active principle (Fig 1)

Authentic bacterial cultures were collected from king institue, guindy, Madras and maintained by periodical subcultures. The bacteria used were: staphylococcus qureus (a), Escherichia coil (b) Proteus mirablis(c) salmonella typhimurium (d) Pseudomonas aeruginosa (e) and Klebsiella aerogenes (f). The disk diameter is 6 mm. The antibacterial activity of the extracts ad their fractions were tested by disk diffusion method in mueller Hinton Agar at 4,2,1 and

0.5 mg/disc. Different fractions showed activity against these bacteria (Fig 2).

RESULTS AND DISCUSSION:

The chloroform extract sowed good activity (>10mm) against E.Coli and P.aeruginosa and minimum activity (8mm) against S. typhimurium and K. aerogenes at 4mg/disk. The hexane eluate showed activity against K. aerogenes (11 cm: 4mg/dics) the benzene eluate was ineffective against all the strains. Benzene-ethylacetate (4:1) eluate showed a good inhibition (715 mm) against E.coli, S.typhimurium and P.aeruginosa.

The alcohol extract of the plat shoed minimum activity (8mm) against P. aeroginosa, S.typhimurium, P. mirablis (4mg/disc). The ethylacetate extractive was active (12mm) against K.aerogenus, n. butanol extractive against P.mirablis (18mm) and S.typhimurium (12mm) and the aqueous portion against P. mirablis (`16 mm), s.typhimurium (12mm) and P.aeroginosa (8mm).

The TLC pattern of benzene-ethylacetate (4:1) eluate of chloroform extract and of ethylacetate extractive of the alcohol extract was similar and therefore mixed ad column chromatographed over silica gel. The benzene: ethylacetate (9:1) eluate showed activity against E.coli (8mm.0.5mg/disc)and ethylacetate eluate against K.aerogenes (10mm. 0.5 mg/disc) std. Neomycin sulphate – could not be preceded further due to paucity of the isolated fractions.

The n-butanol was column chromatographed over silica gel and eluted successively with ethyl acetate ethyl acetate-methanol (9:1) and ethyl acetate methanol (1:1) and the anti bacterial activities eluates was tabulated (Table 1) the ethylacetate eluate was concentrated and triturated wit the same solvent and supernatent solution decanted and concentrated. Addition of few drops of hexane yielded a glycoside, identified as β-Sitosterol – β-D-glycopyranoside which did not exhibit an activity against the tested bacteria.

The residue from the above, on column chromatography over silica gel and elution with ethyl acetate: methanol (9:1) yielded a light brown powder on concentration. This answered tests for phenol and sugar. Its hydrolysis with 5% alcoholic hydrochloric acid yielded a sugar, identified as aglucose by paper chromatography. Its antibacterial activity is given (Table 2) it showed a promising activity at 0.5 mg/disc against P.mirablis (10 mm) but insensitive to S.typhimurium.

The IR spectrum of the compound showed a peak at 1620 cm -1 indicative of aromatic ring and a broad peak at 3440 cm-1 indicative of hydroxyl group. The Uv spectrum showed absorption maxima at 280m and shoulder at 320 nm. The above date suggest that the compound could be a phenolic glucoside. The activity of n-butanol extractive against P.mirablis could be attributed to the presence of the phenolic glucosid4e present in the extract.

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Table 1
Antibacterial activity of n- butanol fraction of alcohol extract

Bacterial strain	Zone of inhibition mm/disc									
	Ethyl acetate (13)			Ethyl acetate Methanol (9:1) (14)			Ethyl acetate Methanol (1:1) (15)			Standard
	1	2	3	1	2	3	1	2	3	
c. Proteus miriabilis	Not done			18	16	14	12	10	NS	10
d. Slmonella typhimurium	12	10	NS	18	16	NS	12	NS	NS	20

Table -2
Antibacterial activity of compound

Bacterial strain	Zone of inhibition mm/disc			
	1	2	3	Std
c. Proteus miriabilis	18	14	10	10
d.Slmonella typhimurium	NS	NS	NS	20

NS: Not sensitive

1: 2mg/disc
2: 1 mg/disc
3: 0.5 mg/disc
Standard: Neomycin 30 mmg/disc

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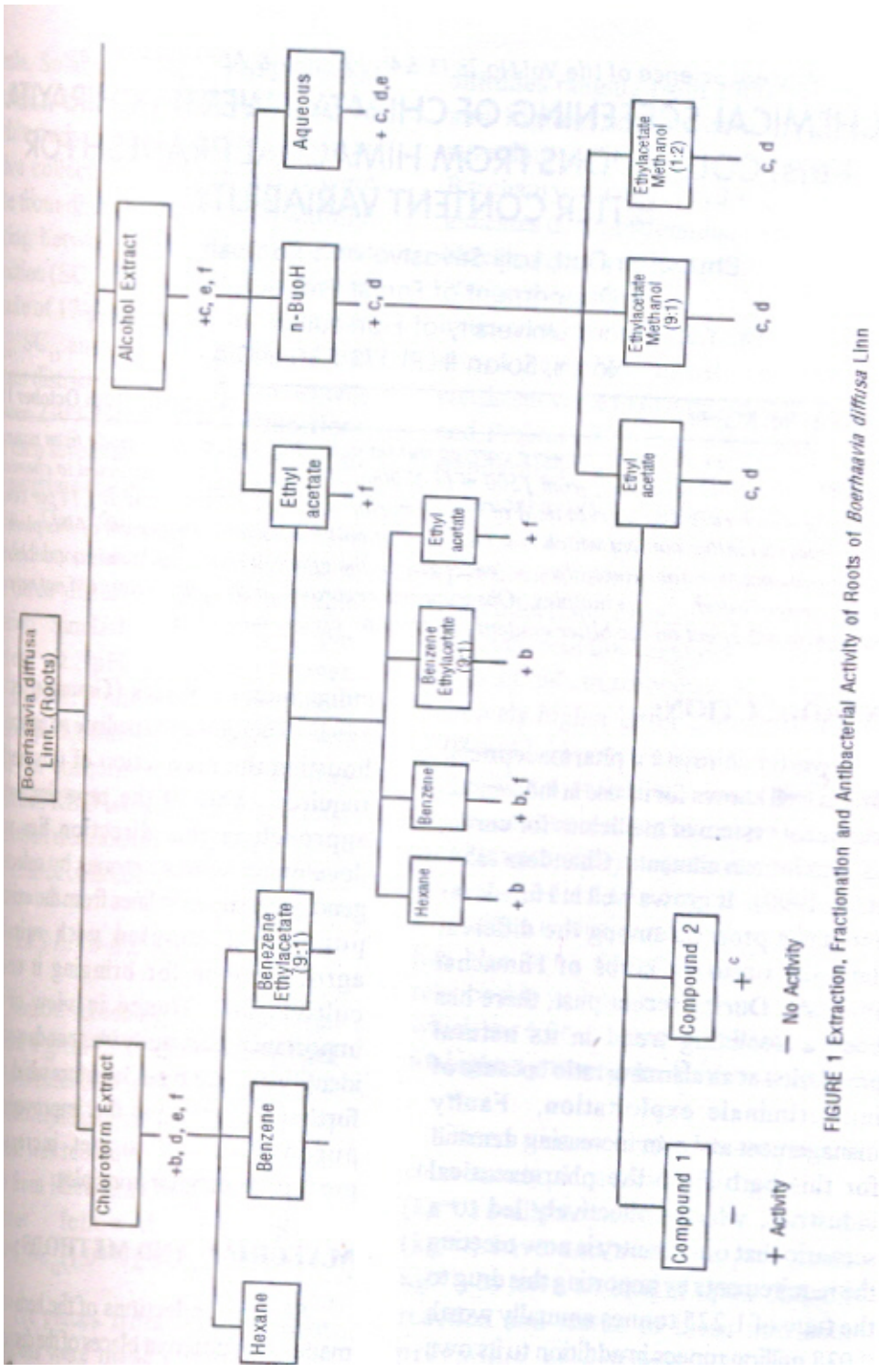

FIGURE 1 Extraction, Fractionation and Antibacterial Activity of Roots of *Boerhaavia diffusa* Linn

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