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GC-MS and Phytopharmacological Analysis of Aqueous Distillate of *Boerhavia diffusa* Roots

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Keywords: *Boerhavia diffusa*, Phytochemical constituents, aqueous distillate, GC-MS analysis.

ABSTRACT

Objective: To investigate the phytochemical constituents of aqueous distillate of *Boerhavia diffusa* roots by using GC-MS technique.Methods: GC-MS analysis of aqueous distillate of *Boerhavia diffusa* roots were performed using a Perkin-Elmer GC Clarus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite -I, fused Silica Capillary Column (30mm x0.25mm1Dx1 µ Mdf, composed of 100 % Dimethylpolysiloxane. Results: In this study results showed the presence of many phytochemical compounds such as phenol, lipid, saponin, flavonoids, steroids, protein, and carbohydrates. In GC-MS analysis, 31 bioactive Phytochemical compounds were identified in the aqueous distillate of *Boerhavia diffusa* roots, the components were identified by comparing their relation indices and mass spectra fragmentation patterns with those stored on the MS-Computer library and also from the published literatures. Stigmast-4-en-3-one 48.066% followed by Cycloartane-3.beta., 25-diol (RT) 46.226%, gamma-Sitosterol with RT 44.193, Stigmasterol with 42.788% RT, Dihydrobrassicasterol with RT 42.074%, Lichesterol RT 41.647%, Cholesteryl chloroformate RT 39.518 % were seem to be the major constituents. Conclusion: From the results, it can be concluded that the aqueous distillate of *Boerhavia diffusa* roots showed 31 bioactive phytochemical constituents. The presence of these constituents justifies the use of plant roots by folk practitioners. Since terpenoids and sterols are the basic components responsible for antioxidant, antibacterial, anti-inflammatory activity and ailments of many diseases, we identify the presence of terpenoids and sterols in aqueous distillate of *Boerhavia diffusa* roots as major component, hence it may be used in various diseases.

INTRODUCTION:

As mentioned in ancient Indian books like *Charak Samhita, Sushruta Samhita, Ras Tantra Sar, Bhavprakasha, Ras Tarang,* Nayan Drastam and *Astanghriday,* Sushruta, the father of Surgery in India before 500 BC researched that, there are a number of plants which are used in various diseases, As juice, extract, kwath, paka, arka etc, either single or in compound formulations with other plants[1]. Medicinal plants have no side effects due to presence of bioactive constituents. Medicinal plants are of great importance to the health of individuals and communities in general[2]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds[3].

In Ayurveda (Indian system of medicine) various disorders and diseases have been described in great details. The World Health Organization (WHO) supports the use of traditional medicine, they are proven to be efficacious and safe[4]. Many of the indigenous medicinal plants are used as spices and food. They also sometimes added to foods meant for pregnant women and nursing mothers for medicinal purposes[1-3]. In addition, the use of herbal medicine for the treatment of diseases and infections is as old as mankind[4].

Boerhavia diffusa is a member of Nyctaginaceae family. *Boerhavia diffusa* is a perennial creeping weed; a number of branches spread out from the node of main stem and cover most of the surrounding area. The stem is usually prostrate and woody. Leaves are simple, ovate or oblong or round in shape flowers are minute, hermaphrodite, subcapitates, pedicellate. Stamens are usually two or three in number and the stigma is peltate. The fruit is achene, ovate, oblong, and pubescent. Five ribbed and glandular and anthocarpous[5, 6, 7]. Taxonomical classification, common names and photographs of leaves, flower and plant of (*Boerhavia diffusa*) is given in figure 1.

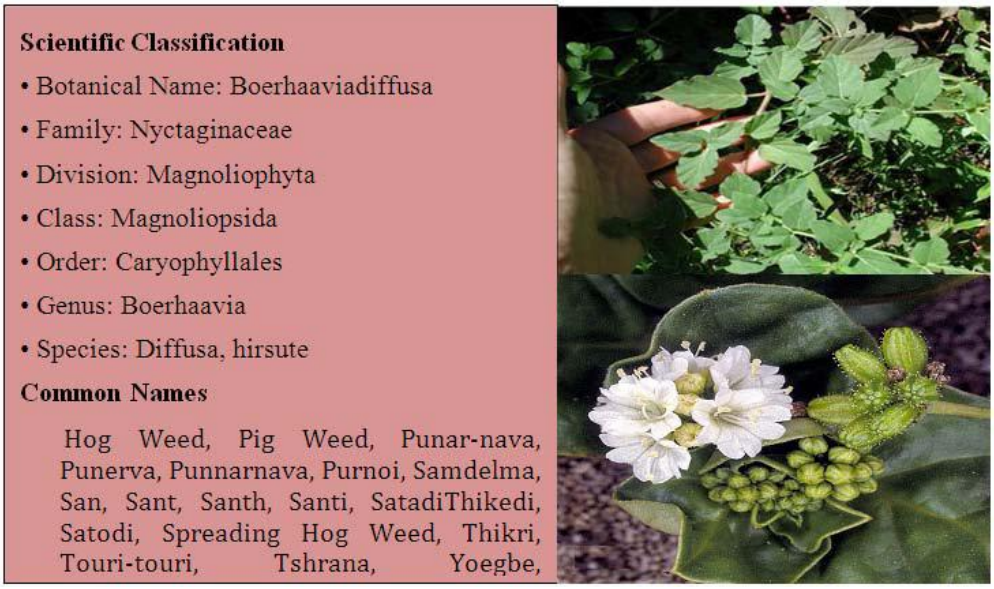

Fig. 1: Taxonomical classification, common names and photographs of leaves, flower and plant of *Boerhavia diffusa*

Part used: Root, Leaves and seeds[7]

GEOGRAPHICAL DISTRIBUTION AND HABITAT:

Genus *Boerhavia*, consisting of 40 species is distributed in tropical and subtropical regions and warm climate. It is found in Ceylon, Australia, Sudan and Malay Peninsula, extending to China, Africa, America and Islands of the Pacific. Among 40 species of Boerhaavia, 6 species are found in India, namely *B. diffusa*, *B. erecta*, *B. rependa*, *B. chinensis*, *B. hirsute* and *B. rubicunda*. *Boerhavia diffusa*, in India, is found in warmer parts of the country and throughout up to 2000 m altitudes in the Himalayan region. It is a perennial, spreading hogweed, commonly occurring abundantly in waste places, ditches and marshy places during rains. The plant is also cultivated to some extent in West Bengal[8, 9, 10, 11].

Boerhavia diffusa is a perennial creeping weed, prostrate or ascending herb, up to 1 m long or more having spreading branches. The plant grows profusely in the rainy season, and mature seeds are formed in October-November. Due to its sticky nature, the plant gets stuck on the clothes of human beings and on the legs of animals, which helps in its dispersal from one place to another[12].

The shape of the leaves varies considerably - ovate-oblong, round, or subcordate at the base and smooth above. Margins of the leaves are smooth, wavy, or undulate. The upper surface of

the leaves is green, smooth, and glabrous, whereas it is pinkish white and hairy beneath. Leaves are up to 5.5 × 3.3 cm2 in area. The seeds germinate before the onset of the monsoon[13].

Flowers are minute, usually fascicled or sub umbellate on the ultimate branchlets, pink, white and about 1.5 mm long. These are hermaphrodite, pedicellate, and white, pink, or pinkish-red in color. Bracts are deciduous and involucrate. A perianth is present in the place of a calyx and corolla, which is tubular in shape, the tube being short and narrow at the base and funnel-shaped at the top and constricted above the ovary. There are five lobes, which are small and acute. Two or three stamens are present and are slightly exerted. The stigma is peltate. The achene fruit is detachable, ovate, oblong, pubescent, five-ribbed and glandular, anthocarpous, and viscid on the ribs[6, 7, 8, 13.]

The taproot is tuberous, cylindrical to narrowly fusiform to conical or tapering, light yellow, brown or brownish gray. It is thick, fleshy and very bitter in taste. Some workers have studied the regeneration of this plant through tissue culture[14]. Roots contained 0.15% alkaloid punarnavine. Increase in levels of indole- acetic acid (IAA) in MS medium reduced the number of roots regenerated from the leaf segment, their length and alkaloid content[15].

The present study clearly demonstrated that the aqueous distillate of *Boerhavia diffusa* roots still possesses some useful phyto compounds analyzed by GCMS, which have many pharmacological properties and may provide maximum information for the use in medicine[16].

MATERIALS AND METHODS:

Collection of plant materials:

Well identified samples of plant *Boerhavia diffusa* Linn. (roots) were collected from their natural habitats along with the samples from local market[17]. The samples have been authenticated by *National Botanical Research Institute, Lucknow.* (SPECIFICATION No: NBRI-SOP-202). The plants were washed in tap water and air dried. Roots were collected in separate paper covers and dried in shade for 15 days[18]. The dried plant materials (roots) of (*Boerhavia diffusa*) made to fine powder using homogenizer. The dry powder obtained was stored at room temperature in airtight containers[19].

METHOD OF PREPARATION:

The drug was cleaned and coarsely powdered. Some quantity of water is added to the drugs for soaking and kept over-night. This makes the drugs soft and when boiled releases the essential volatile principles easily. Powdered root of plant was boiled with distilled water (1:16) where 1 part is drug and 16 parts are distilled water. The following morning it is poured into the distillation assembly and the remaining water was added and boiled. The vapour was condensed and collected in a receiver. In the beginning, the vapour consists of only steam and may not contain the essential principles of the drugs. It should, therefore, be discarded. The last portion also may not contain therapeutically essential substance and was discarded. The aliquots collected in between contain aroma of the active ingredients and may be mixed together to ensure uniformity of the aqueous distillate[8]. Condensed aqueous distillate of roots, were stored in airtight container, and used for GCMS study and preliminary screening of phytochemicals such as alkaloids (Mayer's and Dragendorff's tests), anthroquinones (Borntrager's test), flavonoids (NaOH or Alkaline reagent test), cardiac glycosides (Keller-Killani test), phenols (Phenol test), quinines (NaOH test), reducing sugars (Fehling's test), saponins (Foam test), steroids (Libermann-Burchard test), soluble starch (Iodine test), tannins (gelatin test) and terpenoids (Salkowski test), were carried out following the methodologies of Harbone (1998), Sofowora (1993), Trease and Evans (2002).

Standardization of aqueous distillate

1. Preliminary Phytochemical screening of aqueous distillate for the presence of group of compounds

The aqueous distillate so prepared has been tested qualitatively for the presence of different groups of compounds and details are as below[10].

Table 1: Phytochemical tests:

Preliminary phytochemical tests for *arka* (aqueous distillate) of roots of *Boerhavia diffusa* by different solvents for presence of natural constituents			
Sr. No.	**Natural products**	**Test Performed**	**Result (in triplicate)**
1.	Alkaloid	Dragendorff's test	+ ve
2.	Flavone	Alkaline test	+ve
3.	Steroid	Shinoda test	+ve
4.	Tannin	Liebermann- Burchard reagent	-ve
5.	Sugar	Liebermann- Burchard reagent	-ve
6.	Terpenoid	Liebermann- Burchard reagent	+ve
7.	Saponin	Neutral $FeCl_3$	+ve
8.	Glycoside	Molisch's test	+ve
9.	Glycoside	Noller's test	+ve
10.	Glycoside	NaOH solution	+ve
11.	Glycoside	Borntrager's test	+ve

2. Gas Chromatography-Mass Spectrometry Analysis:

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of aqueous distillate of *Boerhavia diffusa* roots was performed using a Clarus 500 Perkin Elmer gas chromatography equipped with a Elite-5 capillary column [5% Phenyl and 95% methyl Polysaccharides Siloxane] and mass detector turbo mass gold of the compact which was operated in E1 mode. Elite wax (Polyethylene glycol) (30mmx0.25mm X0.25umdf) is a polar column used in the estimation) an insert gas such as Helium is used as a carrier gas at a flow rate of 1ml/min, split 10:1. The components of test sample are evaporated in the injection part of the GC equipment and segregated in the column by adsorption and desorption technique with suitable temperature programmes of the over controlled by software different components are eluted from based on the boiling point of the individual components[20]. The GC column is heated in the oven between 110ºC to 28ºC. The time at which each component eluted from the GC column is termed as retention time (RT). The total GC running time is 36 min. The eluted component is detected by the mass detector. The spectrum of the known components stored in the NIST library and ascertains the name, molecular weight and structure of the components of the test material in GC-MS study.

Identification of components was based on comparison of their mass spectra with those of Wiley and NIST Libraries and as well as on comparison of their retention indices with literature[21, 22].

RESULTS AND DISCUSSION:

The present studies have been carried out in roots of *Boerhavia diffusa* to check the presence of medicinally active constituents. Phytochemical screening of the aqueous distillate indicated the presence of phenol, lipid, saponin, flavonoids, steroids, protein, and carbohydrates qualitatively analyzed and the results are presented in **Table-1**.

In the GC-MS analysis, 31 bioactive phytochemical compounds were identified in the aqueous distillate of *Boerhavia diffusa* roots; Most of them were not reported earlier but screened and characterized in several other plants including their biological activities. GC-MS analysis also depicted that these extreme bioactivities are due to the presence of the different bioactive compounds which are already identified and characterized in different medicinal plants but only few of them are reported[23, 24].

Table 2: Phytochemical compounds identified from aqueous distillate of *Boerhavia diffusa* roots by GC-MS with its pharmacological activities

Peak	Retention Time	Name	Area %	Molecular Formula	Molecular Wt.	Activity
1	3.953	Glycerin	3.45	C3H8O3	92	Humectant [31]
2	4.928	4-Ethyl-o-xylene	2.12	CH4N2O	60	Antioxidant [32]
3	5.085	Eucalyptol	1.05	C10H18O	154	Antioxidant [33]
4	7.566	Isomenthone	0.92	C10H18O	154	Antimicrobial, [34] Antioxidant
5	8.243	Nonylcyclopropane	1.11	C12H24	168	Antibacterial [35]
6	9.344	Hexahydrocumene	0.94	C9H18	126	Anti-inflammatory [36]
7	10.526	2-Dodecanone	0.38	C12H24O	184	Antifungal, Antioxidant [37]
8	12.135	Decane, 5-cyclohexyl-(1-Butylhexyl)cyclohexa	0.59	C16H32	224	Microbicidal, Microbiostatic [38]

		ne)				
9	12.609	1-Tridecene	2.62	C13H26	182	Antioxidant [39]
10	14.259	2-Methoxyphenyl isothiocyanate	2.66	C8H7NOS	165	Antihypertensive [40]
11	14.924	Vacenic acid	2.73	C18H34O2	282	Anticarcinogenic [41]
12	16.670	1-Pentadecene	3.76	C15H30	210	Antimicrobial [42]
13	18.227	Cetylglycidyl ether	5.24	C19H38O2	298	Anti-inflammatory [43]
14	20.353	1-Hexadecanol	2.73	C16H34O	242	Antimicrobial, Topical anti-inflammatory, Anticancer [44]
15	21.779	8-Octadecanone	1.73	C18H36O	268	Anti-inflammatory, Antibacterial [45]
16	22.635	Hexadecanoic acid methyl ester	1.57	C17H34O2	270	Antioxidant [46]
17	23.263	n-Hexadecanoic acid	5.53	C16H32O2	256	Antimicrobial [47]
18	23.331	Dibutyl phthalate	9.78	C16H22O4	278	Purging agent for tumor cell [48]
19	23.698	1-Heneicosanol	1.44	C21H44O	312	Anti HIV, Anti carcinogenic [49]
20	25.329	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	0.90	C19H34O2	294	Antimicrobial [50]
21	25.412	9-Octadecenoic acid, methyl ester	1.63	C19H36O2	296	Antimicrobial, Antioxidant [51]
22	26.026	9-Octadecenoic acid (Z)	2.11	C19H36O2	296	Anti-inflammatory [52]
23	30.074	Pentadecanal	0.62	C15H30O	226	Antimicrobial, Antifungal [53]
24	31.850	Monoethylhexyl phthalate	1.39	C16H22O4	278	Antitumor [54]
25	39.518	Cholesterylchloroformate	1.39	C28H45ClO2	448	Anticancer [54]
26	41.647	Lichesterol	1.98	C28H44O	396	Antifungal [55]

27	42.074	Dihydrobrassicasterol	3.94	C28H48O	400	Antioxidant, Immunomodulator, CNS depressant [55]
28	42.788	Stigmasterol	9.47	C29H48O	412	Antioxidant, Antibacterial [53]
29	44.193	gamma-Sitosterol	20.03	C29H50O	414	Analgesic, Anti-inflammatory, Antioxidant [56]
30	46.226	Cycloartane-3.beta.,25-diol	1.45	C30H52O2	444	Antifungal, Anticancer [56]
31	48.066	Stigmast-4-en-3-one	4.76	C29H48O	412	Hypoglycemic, Antioxidant [56]

The identification of phytochemical compounds in GC-MS is based on the peak area, molecular weight and molecular formula of the compounds[25]. The prevailing compounds in aqueous distillate of *Boerhavia diffusa* roots are, Stigmast-4-en-3-one 48.066% Retention Time (RT) followed by Cycloartane-3.beta., 25-diol (RT) 46.226%, gamma-Sitosterol with (RT) 44.193, Stigmasterol with 42.788% (RT), Dihydrobrassicasterol with (RT) 42.074%, Lichesterol (RT) 41.647%, Cholesteryl chloroformate (RT) 39.518 %. **Figure 2, 3 and 4** shows mass spectrum and structures of Stigmast-4-en-3-one, Stigmasterol and gamma-Sitosterol respectively. This data interpreting the aqueous distillate of *Boerhavia diffusa* roots contains aqueous soluble compounds useful to treat diseases might be the central theme behind using this plant directly as traditional tribal medicine[26].

This is the novel attempt of research on aqueous distillate of *Boerhavia diffusa* roots that make this study novel and useful[19]. In addition, this study provides evidence that the compounds we identified are well characterized in various other rare plants. Getting of rare 31 bioactive compounds in aqueous distillate of this plant for treatment of different diseases is a difficult task[20]. Aqueous distillate of *Boerhavia diffusa* roots is cost effective and safe medicine for the treatment of different ailments[21]. The results are in accord with tribal belief for which they use as traditional medicine for different bioactivities and treatment of ailments[22].

FIG. 1. GC-MS analysis of aqueous distillate (arka) of *Boerhavia diffusa* roots

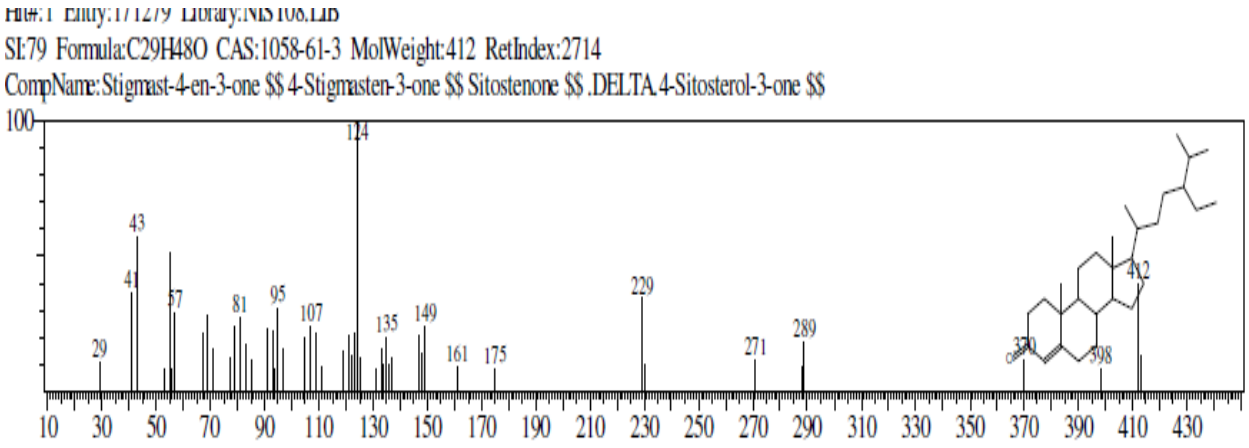

FIG. 2. Mass Spectrum of Stigmast-4-en-3-one

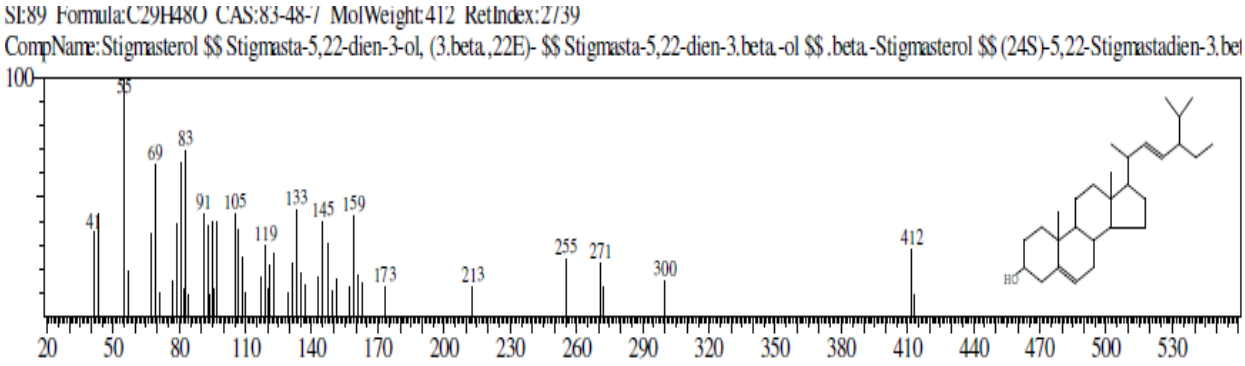

FIG. 3. Mass Spectrum of Stigmasterol

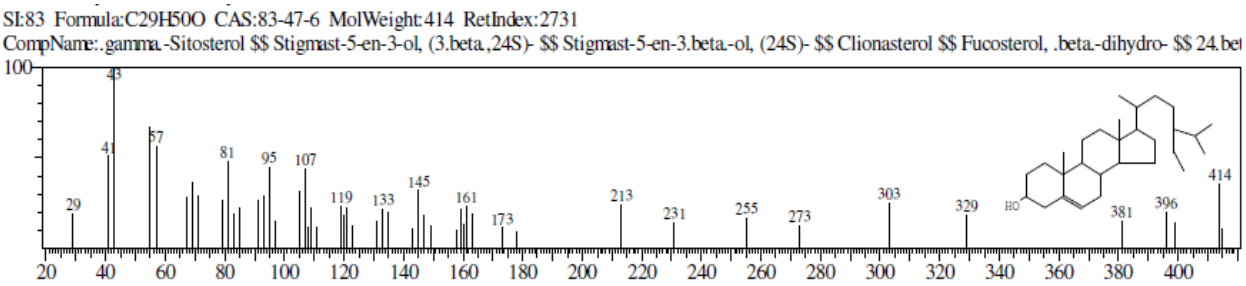

FIG. 4. Mass Spectrum of gamma-Sitosterol

Pharmacological activity with their specific mechanism of action, components identified in GCMS of *Boerhavia diffusa* aqueous distillate

1. Glycerin: Osmotic dehydrating agent, humectant [31]

It is a potent osmotic dehydrating agent with additional effects on brain metabolism. In doses of 0.25-2.0 g/kg Glycerin decreases intracranial pressure in numerous disease states. It is also effective in lowering intraocular pressure in glaucoma and shrinking the brain during neurosurgical procedures. Also, acts as humectant.

2. 4-Ethyl-o-xylene: Antioxidant[32]

Found in post-hydrodistilled *Callitris Columellaris* leaf and reported high antioxidant activity in DPPH free radical scavenging assay.

3. Eucalyptol: Smooth muscle relaxant, antibacterial[33]

Eucalyptol is an essential oil that relaxes bronchial and vascular smooth muscle of male Wistar rats weighing 250 to 300 g. Oil of eucalyptus has antibacterial activity against Gram-positive as well as Gram negative bacteria resistant to commonly used antimicrobial agents.

4. Isomenthone: Antimicrobial[34]

Possess significant antimicrobial activities mainly due to the presence of oxygenated monoterpenes in their chemical composition, The essential oil of *M. longifolia* has shown interesting antimicrobial activity against *Escherichia coli, Salmonella typhimurium*, [14] *Listeria monocytogenes, Aspergillus flavus.*

5. 2-Dodecanone: Antifungal[35]

A possible mode of action may be an irreversible inhibition of an enzyme due to a nucleophilic attack at the 4-position. It inhibited the growth of *T. mentagrophytes* at the same concentration, 25 μg/mL. Activity against this fungus decreased at longer or shorter chain lengths.

6. 2-Methoxyphenyl isothiocyanate: **Antibacterial**[36]

Isocyanates and isothiocyanates are important antibacterial intermediates belonging to the family of compounds known as hetero cumulenes. The chemistry of these molecules is dominated by two modes of reaction: nucleophilic addition and cycloaddition.

7. Vaccenic acid: Cholesterol lowering agent[37]

A 2008 study at the University of Alberta suggests that vaccenic acid feeding in rats over 16 weeks resulted in lowered total cholesterol, lowered LDL cholesterol and lower triglyceride levels.

8.1-Pentadecene: Antimicrobial[38]

Acetone extract of *Spirulina platensis have* 1-Pentadecene showed antimicrobial activity.

9: 1-Tridecene: Antioxidant[39]

Ethyl acetate extract of *Azadirachta indica* 50 µ gm/ml have 1-Tridecene, showed antioxidant activity.

10. 1-Hexadecanol: Antimicrobial[40]

The chemical compositions of the ethanolic extract of leaves and bark of *Naringi crenulata* have, 1-Hexadecanol showed antimicrobial activity.

11. 8-Octadecanone: **Anti-inflammatory activity**[41]

95% ethanol extract of *Bauhinia variegate* leaves have 8-Octadecanone showed anti-inflammatory activity.

12. Hexadecanoic acid methyl ester: Antioxidant[42]

95% methanol extract of *Cassia italica* leaf having Hexadecanoic acid methyl ester, showed Antioxidant, Flavor, Hypocholesterolemic Pesticide, 5-Alpha reductase inhibitor.

13. n-Hexadecanoic acid: **Hypocholesterolemic**[43]

Cassia Italica leaf 95% methanol extract have n-Hexadecanoic acid showed antioxidant, flavor, hypocholesterolemic pesticide, 5-alpha reductase inhibitor.

14. Dibutyl phthalate: Purging agent[44]

Act as purging agent for tumor cell.

15. 1-Heneicosanol: Antioxidant[45]

Ethyl acetate extract of *Phyllanthus emblica* L. bark have 1-Heneicosanol showed antioxidant activity.

16. 9, 12-Octadecadienoic acid (z, z)-, methyl ester: Antimicrobial[46]

Petroleum ether and ethanol leaf extracts from *Abrus precatorius* Linn having 9,12-Octadecadienoic acid (z,z)-, methyl ester, showed antimicrobial action and also effective in coughs, flu, eye infection, inflammation, skin disease, bacterial and viral infections.

17. 9-Octadecenoic acid, methyl ester: Antimicrobial and Antioxidant[47]

Methanol and acetone seed extract of *Cassia glauca* have 9-Octadecenoic acid, methyl ester showed maximum antimicrobial and antioxidant activity.

18. 9-Octadecenoic acid (Z): **Antimicrobial[48]**

Petroleum ether extract of mushroom *Pleurotuseous* is found to have 9-Octadecenoic acid showed antimicrobial activity.

19. Pentadecanal: Antimicrobial[49]

Solanum essential oils have Pentadecanal which possess strong antimicrobial activity in addition to the potent cytotoxic potential of *S. erianthum* leaf oil against Hs 578T and PC-3 cells.

20. Mono-ethyl hexyl phthalate (MEHP): Antitumor[50]

Mono-ethyl hexyl phthalate (MEHP) on MA-10 Leydig tumor cells and in Leydig cells may act as a mitochondrial toxicant and lipid metabolism disrupter.

21. Cholesteryl chloroformate: Brain diseases[51]

Used in the development of high drug-loading nanomicelles targeting steroids to the brain to increase the grafting ratio and drug loading, cholesterol was converted to cholesteryl

chloroformate system may provide a promising carrier to deliver hydrophobic drugs across the blood–brain barrier for the treatment of brain diseases.

22. Lichesterol: Antifungal[52]

The antifungal activity of the methanol extracts of lichens *Hypogymnia physodes* and *Cladonia foliacea* proved due to presence of Lichesterol.

***23.* Dihydrobrassicasterol: Cytotoxic[53]**

Oxidized derivatives of Dihydrobrassicasterol act as cytotoxic and apoptotic potential in U937 and HepG2 cells.

24 Stigmasterol: *Kalanchoe pinnate*[53]

25. Gamma-Sitosterol: Cardiovascular protection[54]

It has been associated with cardiovascular protection, exerting its effect mainly through increasing the antioxidant defense system and effectively lowering the serum cholesterol levels in humans. However, its anti-inflammatory effect on endothelium is unknown.

26. Cycloartane-3.beta., 25-diol: Antitumor[55]

Forty-eight natural and semisynthetic cycloartane-type and related tri terpenoids have been evaluated for their inhibitory effects on Epstein-Barr virus early antigen (EBV-EA) activation induced by the tumor promoter 12-O-tetradecanoyl phorbol -13-acetate (TPA) in Raji cells as a primary screening test for anti-tumor promoters. Exhibited inhibitory effects on skin tumor promotion in an in vivo two-stage mouse skin carcinogenesis test using 7, 12-dimethylbenz [a] anthracene (DMBA) as an initiator and TPA as a promoter.

27: Stigmast-4-en-3-one: Hypoglycaemic[56]

Intravenous administration of the hexane extract of the bark of *Anacardium occidentale* (cashew) in normal, healthy dogs produced a significant lowering of the blood glucose levels. Pursuit of the hypoglycemic principle(s) in the hexane extract resulted in the isolation and characterization of two compounds, stigmast-4-en-3-ol (1) and stigmast-4-en-3-one (2). These compounds were purified by chromatographic methods and the structures were characterized by spectroscopic methods. Both compounds produced significant hypoglycemic

activity after intravenous administration at a dose of 1.3 mg/kg body weight. The bark of the cashew plant, A. occidentale, exhibited a hypoglycemic effect probably due to the presence of stigmast-4-en-3-one.

CONCLUSION:

GCMS study has revealed the presence of many secondary metabolites and bioactive compounds in the roots of *Boerhavia diffusa*[23]. The roots of *Boerhavia diffusa* have been used as a treatment in many disorders. It is found to be rich source of Glycerin, Eucalyptol, Isomenthone, Nonylcyclopropane, Lichesterol, Dihydrobrassicasterol, Stigmasterol, gamma-Sitosterol, 4-Ethyl-o-xylene, Eucalyptol, Isomenthone, Nonylcyclopropane, Hexahydrocumene, 2-Dodecanone, Decane, 5-cyclohexyl- (1-Butylhexyl)cyclohexane), 1-Tridecene, 2-Methoxyphenyl isothiocyanate, Vacenic acid, 1-Pentadecene, Cetylglycidyl ether, Dibutyl phthalate, 1-Heneicosanol, Pentadecanal, Cholesteryl chloroformate, Dihydrobrassicasterol, etc.

Boerhavia diffusa are steroidal lactones and several of them possess significant pharmacological activities. In addition, future studies should lead to synthesis of these complex and fascinating chemical structures and their generics via modification/addition of different functional groups[24]. It is also important to reveal the bio-efficacy of isolated compounds in combination with other herbs or drugs. Moreover, it is also necessary to study the effects and mechanisms of the isolated molecules *in vivo* using suitable higher animals to ensure its potentiality and safety[25]. The present study comprehensively enlists the isolated compounds with their phytopharmacological activity in aqueous distillate[26].

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