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Research Article

PHYTOCHEMICAL CHARACTERIZATION USING VARIOUS SOLVENT EXTRACTS AND GC-MS ANALYSIS OF METHANOLIC EXTRACT OF *WOODFORDIA FRUTICOSA* (L.) KURZ. LEAVES

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ABSTRACT

Objective: The aim of the present study was to characterize the plant for the presence of biologically active phytochemicals using various solvent extracts of leaves and flower samples of *Woodfordia fruticosa* and GC-MS analysis of methanolic leaf extract of the plant.

Methods: In the present investigation, various extracts of the leaf and flower of *Woodfordia fruticosa* were screened for the presence of steroids, reducing sugars, alkaloids, saponins, tannins, flavonoids, terpenoids, anthraquinones, glycosides and Vitamin C/ascorbic acid by standard qualitative test procedures and further this study was extended by analyzing the potent bioactive compounds in the methanolic extract of *Woodfordia fruticosa* leaves using GC-MS analysis.

Results: In the qualitative phytochemical screening/characterization using various solvent extracts of plant, it was found that most of the biologically active phytochemicals were present in the methanolic extract of *Woodfordia fruticosa* leaves. The GC-MS analysis revealed the presence of twenty one compounds in the methanolic leaf extract of *Woodfordia fruticosa*. The major costituents were Di-N-Octyl Phthalate; Dibutyl phthalate; Hydrocinnamic acid; 3,5-bis(1,1-dimethylethyl)-4-hydroxy- ; 2-Propanol, 1-(2-butoxyethoxy)- and Caryophyllene Oxide, along with other minor constituents.

Conclusion: Results confirmed the presence of therapeutically potent compounds in the leaf extract predominantly tannins and terpenoids.

Keywords: *Woodfordia fruticosa*, Biologically active, Phytochemicals, GC-MS analysis.

INTRODUCTION

***Woodfordia fruticosa* (Linn.) Kurz**. is a rare, much branched, beautiful shrub, with fluted stems and long, spreading branches, 1-3 m high, rarely upto 7 m, commonly occurring through out North India, ascending to an altitude of 1,500 m in the Himalayas, but rather scarce in South India. It is widely cultivated as an ornamental shrub. It is cultivated in gardens for its flowers, which are borne during the summer months. The flowers are flame coloured, hence the name is Fire flamed bush, and yield a red dye used to color fabrics.

Fire flamed Bush (*Woodfordia fruticosa* (L.) Kurz.), commonly called as Dhavari, Dhatki, etc. is a plant with medicinal properties and belongs to the family Lythraceae. Flowers of this plant are the most effective fermentation agents in ayurvedic medicines [1]. It is used both internally as well as externally. The dried flowers of this plant are reported to be used for the treatment of hemorrhoids, dysentery, diarrhoea, liver diseases, piles, disorders of mucous membranes, leucorrhoea, menorrhagia, ulcers, wounds, burning sensations, skin diseases, fever, headache, herpes, etc. [2]. They are often added to the Ayurvedic Arishtas to cause alcoholic fermentation [3]. Externally, the powder of dried flowers is sprinkled on the wounds to alleviate the burning sensation, arrest bleeding and to promote healing. The juice of its fresh flowers applied on the forehead, reduces headache, especially due to pitta. To facilitate dental eruption in children, the powder of its dried flowers is massaged on the gums. The leaves of *Woodfordia fruticosa* possess antibiotic activity *in vitro* against *Micrococcus pyogenes* var. *aureus* as well as sedative properties.

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc. Thus, the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to drug discovery and development. Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance, to make the best and judicious use of available natural wealth. Hence, the present investigation was carried out to determine the possible phytochemical components from *Woodfordia fruticosa* by GC-MS analysis. In recent years, interest for the characterization of organic compounds from plants has been developed. Therefore, an attempt was made to screen and isolate the bioactive compounds, evaluate the bioactive potential and characterize them by GC-MS analysis.

MATERIALS AND METHOD

Plant Material

The plant samples were collected from Haridwar, Rishikesh and Jhadol (Udaipur) and voucher specimens authenticated and deposited in the Rajasthan University's Herbarium (RUBL20635). They were established in the nursery of Department of Botany, University of Rajasthan and used for the study.

Preparation of plant extracts

The plant material (leaves and flowers) of *Woodfordia fruticosa* was collected and washed with water to remove dust and sand, shade dried at room temperature. Extracts were prepared by the method of [4]. The dried plant materials were grounded into fine powder in an electric blender and subsequently sieved for obtaining fine powder. Thereafter, 3 gm of fine powdered sample was weighed and soaked separately in 15 ml of different solvents (Ethyl acetate, Methanol, Benzene, Ethanol and Chloroform) in the ratio of 1:5 weight by volume (w/v). These were allowed to stand for 24 hrs at ambient room temperature. The soaked plant powder was filtered and used as crude extract. Different crude extracts of this plant were stored in a refrigerator and used as such for qualitative phytochemical analysis.

Phytochemical characterization

Crude extracts of the plants were prepared and stored in a refrigerator and used for the phytochemical tests. These extracts were tested for the presence of various bioactive compounds which are given below:-

Test for Flavonoids

To one ml of the extract, a few drops of dilute sodium hydroxide was added. An intense yellow color was produced in the plant extract, which became colorless on addition of a few drops of dilute acid, indicating the presence of flavonoids.

Test for Glycosides

To the test solution in alcohol, 1 ml concentrated H_2SO_4 was added and after hydrolysis of the test solution the presence of sugar was determined with the help of Fehling's solution. A black-red precipitate indicated the presence of glycosides.

Test for Reducing Sugars

To the test solution, 2ml of Fehling's reagent was added followed by 3ml of water, formation of Red-Orange color showed the presence of reducing sugars.

Test for Sterols/Terpenes

Salkowski Test

Extract was treated in chloroform with few drops of conc. sulfuric acid, shaken well and allowed to stand for some time, red color appeared at the lower layer indicating the presence of steroids and formation of yellow colored lower layer indicated the presence of triterpenoids.

Test for Tannins

To the test solution, water and 2ml of 5% $FeCl_3$ was added, formation of blue-black precipitate indicated the presence of tannins.

Test for Anthraquinones

Five ml of the extract solution was hydrolysed with conc. H_2SO_4 extracted with benzene. 1 ml of dilute ammonia was added to it. Rose pink coloration suggested the positive response for anthraquinones.

Test for Alkaloids

Test solution was taken with 2 N HCl. Aqueous layer formed was decanted, to which one or few drops of Mayer's reagent (Potassium mercuric iodide solution) was added. White precipitate or turbidity formed showing the presence of alkaloids.

Test for Saponins

The extract was diluted with distilled water and it was agitated for 15 minutes. The formation of layer of stable persistent foam/froth showed the presence of saponins.

Test for Vit C/Ascorbic acid

To the test solution, 2 ml of water, 0.1 gram of sodium bicarbonate and about 20 mg ferrous sulphate was added, shaken and allowed to stand. A deep violet color was produced. To this 5 ml of 1 M sulphuric acid was added, the color disappeared showing the presence of Vitamin C/ascorbic acid.

Gas Chromatography - Mass Spectrometry (GC/MS) analysis

For the identification of metabolites showing antibacterial and antioxidant potentials, the samples were subjected to GC-MS analysis. The identification of metabolites in the samples was carried using a QP2010 gas chromatography with Thermal Desorption System TD 20 coupled with mass spectroscopy (Shimadzu). The ionization voltage was 70 eV. Gas chromatography was conducted in the temperature programming mode with a Restek column (0.25 mm, 60 m; XTI-5). The initial column temperature was 80^{0}C for 1 min, then increased linearly at 7 ^{0}C min−1 to 220 ^{0}C, hold for 3 min followed by linear increased temperature 10^{0}C min^{-1} upto 290^{0}C hold for 10 min. The temperature of the injection port was 290 ^{0}C and the GC/MS interface was maintained at 290^{0}C. The samples were introduced via an all-glass injector working in the split mode, with helium carrier gas flow rate was 1.2 ml min^{-1}. The identification of components was accomplished by comparison of retention time and fragmentation pattern, as well as with mass spectra in the NIST spectral library stored in the computer software (version 1.10 beta, Shimadzu) of the GC-MS. The relative percentage of each extract constituent was expressed as percentage with peak area normalization.

Identification of components

The identity of the components in the extracts was assigned by the comparision of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literature. NIST08.LIB [5], WILEY8.LIB [6], PESTEI_3.LIB, and FA_ME.LIB library sources were used for matching the identified components from the plant material.

RESULTS

Phytochemical characterization

Results are presented in Table 1. In the qualitative phytochemical screening/characterization using various solvent extracts of plant, it was found that most of the biologically active phytochemicals were present in the methanolic extract of *Woodfordia fruticosa* leaves. In other words, the results confirmed the presence of therapeutically potent compounds in leaf extract of *Woodfordia fruticosa*. It revealed that tannins and terpenoids were predominantly found in all the five extracts, followed by flavonoids which were found in four extracts and anthraquinone, saponins, glycosides and vitamin C /ascorbic acid were found in three extracts. Alkaloids were not found in all the five extracts used for phytochemical screening.

The ethyl acetate extract of flower of *Woodfordia fruticosa* showed the presence of terpenoids, tannins, flavonoids and vitamin C/ ascorbic acid. Methanol extract of flower showed the presence of tannins, terpenoids, flavonoids, reducing sugar, glycosides, anthraquinone, saponin and vitamin C/ascorbic acid. The benzene extract of flower exhibited the presence of anthraquinone, tannins, terpenoids, reducing sugar and flavonoids. Flavonoids, tannins, terpenoids, glycosides and vitamin C/ascorbic acid were found in ethanol extract. Flavonoids and terpenoids were screened in chloroform extract of flower. Glycosides were found in methanol and ethanol extract only and reducing sugar only in methanol and benzene extract. Saponin found only in methanol extract and vitamin C/ascorbic acid only in ethyl acetate, methanol and ethanol extract. Alkaloids were not found in all the five extracts used for phytochemical screening.

GC-MS: Phytocomponents in methanolic extract of *Woodfordia fruticosa* by GC-MS report

The GC-MS analysis revealed the presence of twenty one compounds from the methanolic leaf extract of *Woodfordia fruticosa*. The major costituents were Di-N-Octyl Phthalate (RT: 28.268); Dibutyl phthalate (RT: 22.615); Hydrocinnamic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy- (RT: 22.337); 2-Propanol, 1-(2-butoxyethoxy)- (RT: 24.668) and Caryophyllene Oxide (RT:16.977) (Table 2), along with other minor constituents were also present. The GC-MS chromatogram (Fig. 1) shows the peak area separation. The nature of the chemical compound and its therapeutic activity is depicted in Table 3.

Table 1: It shows qualitative preliminary phytochemical screening/characterization of *Woodfordia fruticosa*.

	Flavonoids	Glycosides	Reducing Sugar	Terpenoids	Tannins	Anthraquinone	Saponin	Alkaloids	Vitamin C/Ascorbic acid
Ethyl acetate	+ +	--	--	+	+	-	-	--	++
Methanol	+	+	+	+	+	+	+	-	+
Benzene	+	-	+	+	+	-	-	-	-
Ethanol	+	+	-	+	+	+	+	-	+
Chloroform	-	+	-	+	+	+	+	-	-

Table 2: Shows GC-MS analysis of methanolic extract of leaf of *Woodfordia fruticosa*

S. No.	Retention time (RT)	Molecular weight	Peak Area (%) (RA)	Name of the compound
1	16.032	180	3.09	Dihydroactinidiolide
2	16.977	220	5.43	Caryophyllene Oxide/Caryophyllene Epoxide
3	17.890	250	2.00	8,11,14-Eicosatrienoic acid / Homo-gamma-linolenic acid
4	17.961	374	2.27	10,12-Pentacosadiynoic acid
5	18.307	346	1.37	6,9,12,15-Docosatetraenoic acid, methyl ester
6	20.285	168	5.20	1-Cyclohexene-1-methanol, .alpha.,2,6,6-tetramethyl-
7	21.366	278	3.62	Diisobutyl phthalate
8	21.999	284	1.77	Hexadecanoic acid,15-methyl-, methyl ester
9	22.144	276	2.84	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
10	22.337	292	6.38	Benzenepropanoic acid / Hydrocinnamic acid/ 3,5-bis(1,1-dimethylethyl)-4-hydroxy-
11	22.615	278	6.89	Dibutyl phthalate/ Butyl phthalate/ Benzene-1,2-dicarboxylic acid di-n-butylester
12	22.831	340	1.82	Eicosanoic acid, ethyl ester/Arachidicacid
13	24.091	282	1.13	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester
14	24.255	296	1.94	Phytol
15	24.668	176	5.61	2-Propanol, 1-(2-butoxyethoxy)-
16	24.775	310	1.99	Ethyl Oleate $$ 9-Octadecenoic acid (Z)-
17	27.622	260	3.62	2H-1-Benzopyran-2-one
18	28.268	390	31.18	Di-n-octyl phthalate $$ 1,2-Benzenedicarboxylic acid
19	29.641	344	2.34	Benzene, 1-[[4-(4-butylcyclohexyl)phenyl]ethynyl]-2,4-dimethyl- $$ 1-([4-(4-Butylcyclohexyl)phenyl]ethynyl)-2,4-dimethylbenzene # $$
20	30.726	204	4.67	gamma.-Elemene
21	32.265	272	4.83	(E,E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene/β-Springene

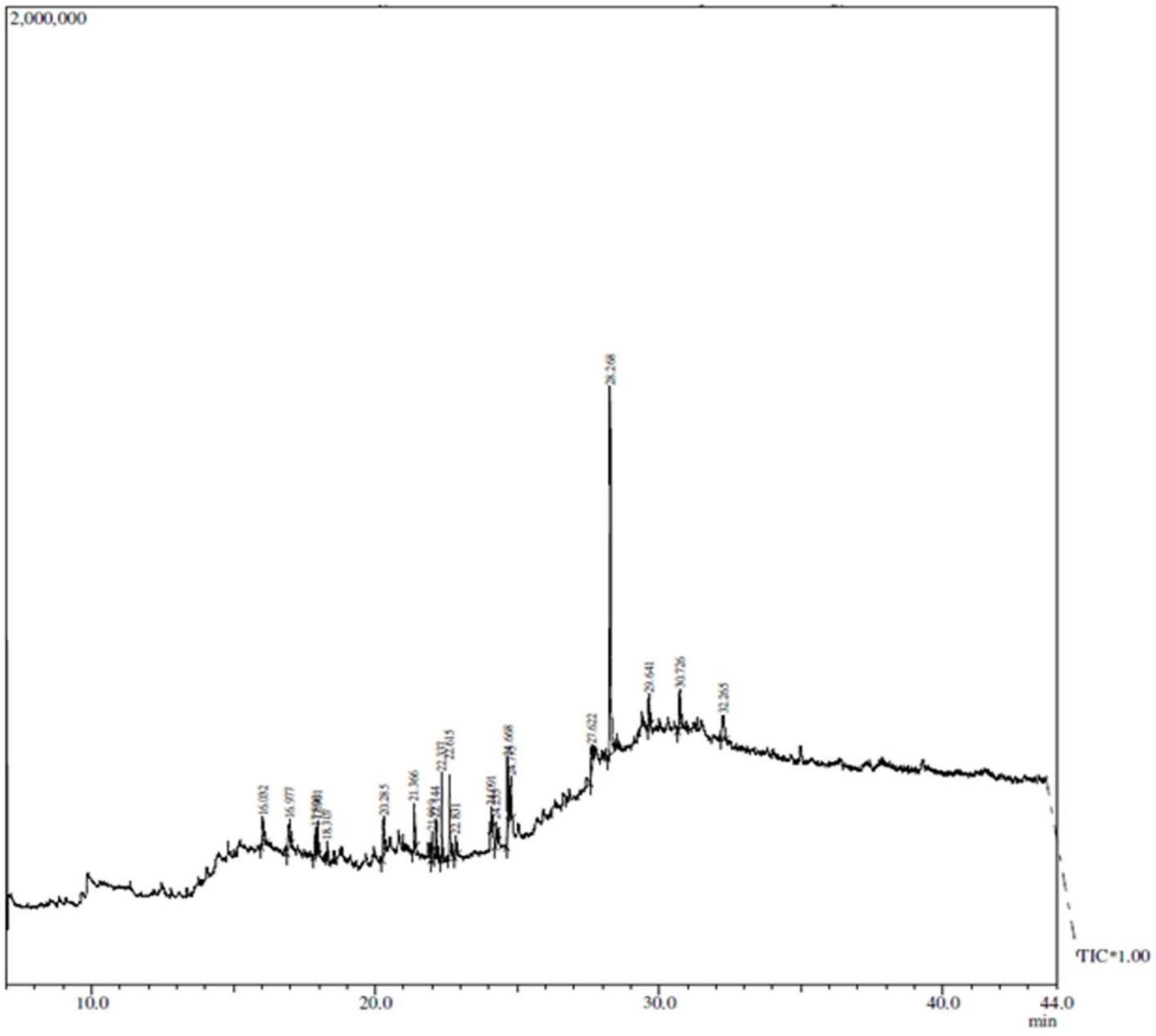

Fig. 1: Shows GC-MS Chromatogram of methanolic leaf extract of *W.fruticosa*

Table 3: Shows activities of phyto-components identified in *W.fruticosa* leaf extract by GC-MS.

RT	Name of the compound	Compound nature	Activity
16.032	Dihydroactinidiolide	Volatile terpene	• used as a fragrance; • a pheromone for a variety of insects
16.977	Caryophyllene Oxide/ Caryophyllene Epoxide	Oxygenated terpenoid,	• well known as preservative in food, drugs and cosmetics; • antifungal against dermatophytes; Anti-tumor, analgesic, antibacterial, anti-inflammatory, sedative.
17.890	Gamma-linolenic acid	An essential polyunsaturated omega-6 fatty acid	• Astringent; anti-inflammatory; anticoagulant properties; reduces liver damage; effective in killing **cancer** cells and treating **rheumatoid arthritis**
18.307	6,9,12,15-Docosatetraenoic acid	a naturally occurring polyunsaturated fatty acid (ω-6 fatty acid)	• Anti cholesterol compound
21.366	Diisobutyl phthalate	an odorless and colorless to faint yellow oily liquid. Phthalates are industrial chemicals that are added to plastics to impart flexibility and resilience and are often referred to as ***plasticizers*.**	• Used in nitrocellulose plastic, nail polish, explosive material, lacquer manufacturing and used with methyl methacrylate applications; • plasticisation of PVC to the production of paints, printing inks and adhesives; • a component of industrial adhesives and catalyst systems for polypropylene and fibreglass manufacture.
21.999	Hexadecanoic acid/ Palmitic acid,	a saturated fatty acid that is the major fat in meat and dairy products	• Lubricant, Antiandrogenic, Flavor, Hemolytic, Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, 5-Alpha reductase inhibitor.
22.144	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	a synthetic, steroidal anti - mineralocorticoid agent	• Additional anti-androgen and weak progestogen properties, as well as some indirect estrogen and glucocorticoid effects, which is used primarily as a diuretic and antihypertensive. • used primarily to treat heart failure, ascites in patients with liver disease, low-renin hypertension, hypokalemia, secondary hyperaldosteronism (such as occurs with hepatic cirrhosis), and Conn's syndrome (primary hyperaldosteronism), • frequently used to treat a variety of cosmetic conditions including hirsutism, androgenic alopecia, acne, and seborrhea in females and male pattern baldness
22.337	Benzenepropanoic acid / Hydrocinnamic acid	carboxylic acid belonging to the class of phenylpropanoids	• used for flavoring, food additives, spices, fragrance, and medicines as it acts as a fixative agent, or a preservative and maintain the original aroma quality of frozen foods. • Act as an antioxidant to prolong shelf life foods, used as a sweetener as well to sweeten food, act as an emulsifier, to keep oil and water mixtures separated, provides flavorings for ice cream, bakery, and confectionary. • used frequently in cosmetic products such as perfumes, bath gels, detergent powders, liquid detergents, fabric softeners, and soaps as it gives off a floral scent.
22.615	Dibutyl phthalate/ Butyl phthalate	a commonly used plasticizer.	• used as an additive to adhesives or printing inks. • also used as an ectoparasiticide
22.831	Eicosanoic acid, ethyl ester/Arachidicacid	the saturated fatty acid with a 20-carbon chain	• It is as a minor constituent of peanut oil (1.1%–1.7%)[2] and corn oil (3%). • used for the production of detergents, photographic materials and lubricants.
24.091	Cyclopropaneoctanoic acid	Alicyclic fatty acids occur naturally in plants, especially certain seed oils, and microorganisms, but only rarely in animal tissues	• can be formed as artefacts from conventional unsaturated fatty acids during food processing • is also used in the treatment of some bacterial infections. Due to its relatively short chain length it has no difficulty in penetrating fatty cell wall membranes, hence its effectiveness in combating certain lipid-coated bacteria, such as *Staphylococcus aureus* and various species of *Streptococcus*.
24.255	Phytol	acyclic diterpene alcohol	• can be used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K1. • Antimicrobial, anticancer, anti-inflammatory, diuretic.
24.775	Ethyl Oleate	fatty acid ester formed by the condensation of oleic acid and ethanol	• used as a solvent for pharmaceutical drug preparations involving lipophilic substances such as steroids • used as a lubricant and a plasticizer • has been identified as a primer pheromone in honeybees • it is used by compounding pharmacies as a vehicle for intramuscular drug delivery, in some cases to prepare the daily doses of progesterone in support of pregnancy.

27.622	2H-1-Benzopyran-2-one	Coumarin - fragrant organic chemical compound in the benzopyrone chemical class, which is a colorless crystalline substance	• has been used in perfumes since 1882 • used as an aroma enhancer in pipe tobaccos and certain alcoholic drinks, although in general it is banned as a flavorant food additive • is used in the pharmaceutical industry as a precursor molecule in the synthesis of a number of synthetic anticoagulant pharmaceuticals • has clinical medical value, as an edema modifier • also used as a gain medium in some dye lasers, and as a sensitizer in older photovoltaic technologies.
28.268	Di-n-octyl phthalate	an aromatic dicarboxylic acid- Plasticizer compound	• Antimicrobial, Antifouling
30.726	gamma.-Elemene	sesquiterpenes	• contribute to the floral aromas of some plants, and are used as pheromones by some insects. • has anti-proliferative effects toward some cancer cell types, indicating the possibility of its use in chemotherapy
32.265	β-Springene	an aliphatic and hydrocarbonic diterpene	• a scent obtained from the dorsal glands of springboks.

DISCUSSION

There is an increasing interest in the phytochemical compounds, which could be relevant to their nutritional incidence and their role in health and disease [7]. In recent years, the interest for the study of the organic compounds from plants and their activity has increased. The combination of an ideal separation technique (GC) with the best identification technique (MS) made GC–MS an ideal technique for qualitative and quantitative analysis for volatile and semi-volatile compounds. The aim of the present study was to develop a rapid method for the quantitative determination of organic compounds in plant and to confirm the phytochemicals present in the plant extracts. Diversity of medicinal plants and herbs containing various phytochemicals with biological activity can be of valuable therapeutic key. Different phytochemicals have been found to have a broad range of activities, which may help in protection against chronic diseases [8].

In the present investigation, the GC-MS analysis revealed the presence of twenty one compounds from the methanolic leaf extract of *Woodfordia fruticosa*. The presence of phyto-components reveals the importance of the plant as medicinally used. So, it is recommended as a plant of phyto - pharmaceutical importance, however, further studies will need to be undertaken to ascertain fully its pharmacological activity. From our investigation, the results confirm the presence of therapeutically potent compounds in leaf extract of *Woodfordia fruticosa*. Similar to our study, [9] also characterized two major compounds through GC-MS analysis of *Rhinacanthus nasutus* leaves; [10] characterized thirteen compounds from methanolic leaf extracts of *Naringi crenulata* ; [11] identified fourteen compounds by GC-MS analysis in *Caralluma fimbriata* ; [12] identified twenty chemical compounds from ethanolic extract of *Mussaenda frondosa*; [13] found abundance of phyto-components, such as alkaloids and majorly flavonoids ; [14] identified twelve chemical constituents from ethanolic extract of aerial parts of *Albizia rocera* (Roxb,) Benth. by GC-MS analysis; [15] identified the presence of atleast thirteen compounds from the ethanolic extract of *H. enneaspermus* .

CONCLUSIONS

From the present study, it can be concluded that most of the biologically active phytochemicals were present in the methanolic extract of *Woodfordia fruticosa* leaves. In other words, the results confirmed the presence of therapeutically potent compound in leaf extract of *Woodfordia fruticosa*.

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