IJPSR (2020), Volume 11, Issue 6 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 July 2019; received in revised form, 22 February 2020; accepted, 25 February 2020; published 01 June 2020

PRELIMINARY PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF METHANOLIC LEAF EXTRACT OF *TEPHROSIA FALCIFORMIS* RAMASWAMI FROM INDIAN THAR DESERT

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Keywords:

Tephrosia falciformis, Methanolic extract, Phytochemicals, GC-MS

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ABSTRACT: Present study was conducted to identify and characterize the phytoconstituents of methanolic extract of leaves of *Tephrosia falciformis* (Pers.) Ramaswami of family Fabaceae. The methanolic extract of leaves was subjected to qualitative screening for primary and secondary metabolites as per standard methods; further GC-MS analysis was carried out for the identification of secondary metabolites. The preliminary analysis of leaf extract confirmed the presence of primary as well as secondary phytoconstituents such as phenolic compounds, alkaloids, flavonoids, terpenoids, phytosterols, *etc*. in methanolic extract. GC-MS analysis of extract revealed the presence of 53 phytochemicals. The presence of various compounds with different chemical groups like quinolene, piperdinone (alkaloids), Neophytadiene (sesqueterpenens), phytol (diterpene alcohol), sitosterol, stigmasterol (sterols) and many fatty acids including their derivatives in considerable amount gives the good prospect for the use of this plant in pharmaceutical preparations to cure health-related problems.

INTRODUCTION: Every plant is rich in phytochemical compounds; consequently, they are a potential source of drugs, but a biological screening is essential to know more about the activities of these compounds. The plant contains phytochemicals in form of primary and secondary metabolites. Primary metabolites are involved in vital metabolic pathways, whereas the secondary metabolites accomplished non-vital functions in plant. Secondary constituents are involved in chemical defense against pathogens and predators and assists in pollination and dispersal. They also act as photoprotectants and allelopathic agents.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(6).3040-46

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(6).3040-46

Activities of these constituents are also beneficial to humans in prevention of diseases in the form of medicine. The important bioactive phytochemicals include alkaloids, phenols, flavonoids, terpenes, steroids, and glycosides. Family Fabaceae is the third-largest family of flowering plants. It consists of 650 genera and 18000 species [1]. Genus *Tephrosia* of this family has 400 species throughout the world [2]. About twenty-seven species of *Tephrosia* are reported in India [3]. This genus is broadly distributed in tropical, subtropical and arid regions of world [4]. Plants of this genus are herb to undershrub and grow like a weed.

Genus *Tephrosia* is well known for its richness in bioactive compounds, especially in flavonoids [5 6]. This genus has been used for the treatment of syphilis, stomach ache, dropsy, inflammation, rheumatic pain and respiratory disorders and as an abortifacient, diuretic laxative also [7, 8].

Tephrosia falciformis (Pers.) Ramaswami, commonly known as rati biyani [9] is 3-4 feet high under shrub grown in sandy plains. It is a rare and threatened plant species of Rajasthan, especially in the Indian Thar desert region **Fig. 1**. The leaves are imparipinnate with 7-9 pairs of leaflets. Both surfaces of leaflets are densely covered with silky grey hair.

This plant is medicinally underexplored, earlier some phytochemicals reported from the seeds of its pods namely triacontanol, Pongamol, Sitosterol, Lanceolatin-B and Lanceolatin-A along with two flavanoid namely Falciformin (7methoxy-8-(3-hydroxy-3-methyl-but-1-enyl) and 7-hydroxy-8-(γ,γ-dimethylallyl) [10, 11]. Any phytoconstituents is not reported from leaves previously, but the present study first time revealed the presence of primary and secondary metabolites in leaf extract of *T. falciformis.*

FIG. 1: *T. FALCIFORMIS*: GROWING IN NATURAL HABITAT

MATERIALS AND METHODS:

Plant Collection: The fully matured fresh leaves of *T. falciformis* (Pers.) Ramaswami was collected from Jaisalmer district of Rajasthan, India in August. The identification of plant was done by taxonomist of BSI, Jodhpur with authentication no.BSI/AZRC/I.2012/Tech/2019-20 (PI.Id)/526. Collected leaves were washed with distilled water and dried in shade at room temperature for 15 days. Dried leaves were coarsely powdered with the help of a grinder. Leaf powder was stored in airtight containers for phytochemical analysis.

Preparation of Crude Extract: About 5 gm dried leaf powder was weighed using an electric balance and extracted into 100 ml methanol of HPLC grade for 72 h with frequent shaking. The extract was filtered through a muslin cloth and Whatman no. 1 filter paper, which was followed by centrifugation for 20 min on 2000 rpm. The extract was again filtered by Whatman no. 1 filter paper and left to evaporate on room temperature till crude extract obtained. The crude extract was transferred into sterile, airtight containers, which were stored in the refrigerator for further use [12].

Preliminary Phytochemical Screening of Leaf Powder: Preliminary phytochemical screening of methanolic extract was done as per the standard methods [13, 14] to prove the presence of phyto-chemicals.

GC-MS Analysis of Extract: The extract was also subjected to GCMS analysis to find out the bioactive compound of the leaf. The sample was prepared by reconstituting crude extract in methanol at the concentration of 1 mg/ml. The gas chromatography-mass spectroscopy (GC-MS) analysis of leaf extract was done on Shimadzu QP-2010 plus system with a thermal desorption system.

GC system was equipped by a fused silica capillary column having dimensions of 30m × 0.25mm × 0.25µm. Helium gas (99.99%) was used as carrier gas at a constant flow rate of 1.21 ml/min. in the split ratio 10:0.An injection volume of 1 µl of the sample was injected into the column. The pressure was kept at 69.0 k Pa. Ionization energy was set on 70Ev. The column oven temperature was initially set on 50 ºC to withhold time of 3 min; the oven temperature was increased to 280 ºC at the rate of 10º withhold time of 24 min. For GC program, Ion source temperature and interface temperature were 220 ºC and 270 ºC respectively. The total running time for GC-MS was 60 min.

The sample was injected in splitless mode and analyzed in MS full scan mode with start m/z 50 & end m/z 650 with a scan speed of 1250.

Interpretation on unknown mass spectrum GC-MS was done by comparing the fragmentation patterns of the mass spectra with the known, and standard compound provided in the database of NIST 16 (National Institute of Standard and Technology) and Wiley 8 library. The compound was identified by their GC retention time. The relative percentage of the amount of each compound was obtained by comparing the average peak area with the total

peak area. The name, molecular formula and molecular weight of each detected compound were determined.

RESULTS: Preliminary phytochemical screening reveals the presence of primary and secondary metabolites. Results of preliminary phytochemicals screening are shown in **Table 1**.

TABLE 1: QUALITATIVE PHYTOCHEMICAL SCREENING OF METHANOLIC LEAF EXTRACT OF *T. FALCIFORMIS*

S. no.	Phytochemical constituents	Test	Methanolic Extract
1	Carbohydrates	Fehling's Test	
		Molisch's Test	+
2	Amino acids	Ninhydrin test	
		Xanthoproteic Test	+
3	Alkaloids	Dragendorff's Test	
		Wagner's Test	+
4	Phenols	$FeCl_3$ Test,	
		Lead acetate Test	+
5	Flavanoids	Shinoda Test,	
		Alkaline reagent Test	+
6	Phytosterols and Terpenoids	Libermann Burchard's Test, Salkowski Test	+
7	Glycosides	Keller killani Test	+
		Glycosides Test	
8	Saponin	Foam Test,	+
		Olive oil Test	
9	Gum and Mucilage	Alcohol Test	
		Ruthenium Red Test	+

Secondary phytoconstituent reported in GC-MS analysis are shown in **Table 2** with peak area percentage, molecular weight and molecular formula. Bioactivity of some significant compounds with molecular structures listed in **Table 3** and chromatogram in **Fig. 2**.

The GC-MS chromatogram of leaf extract of *T. falciformis* revealed the presence of 53 phyto chemicals, which includes alkaloids, terpens, sterols, phenols, flavonoids, and essential oils *etc*. Among these phytochemicals 6-(2-ethoxyphenyl)-5-nitro-2-piperidinone (23.86%) exibits the highest peak percentage followed by 9,12,15-Octadeca-trienoic acid, (Z,Z,Z)- (14.34%), n-Hexadecanoic acid (13.89%),2-Phenyl-4-anilino-6[1H]-pyrimidi-none (10.04%), gamma.-Sitosterol (3.18%), naph-thalene(2.30%), Phytyl tetradecanoate (2.21%), Stigmasterol (1.68%), 1h, 3h-furo[3,4-c]furan, 1,4-bis (3,4-dimethoxyp (1.48%), 2-(3-methoxy-5-methyl-benzyliden)-5,7-dimeth (1.31%),3,6-hepta-nooxepin-4,5-dicarbonsaure-dimeth (1.29), [1] ben-zothieno [2, 3-c]naphtho[1,2-g]quinoline (1.14%), Phytol (1.01%). Lowest peak with 0.18% was reported for two compounds2-Hydroxy-1-(1'-pyr-rolidiyl)-1-buten-3-one and 1,d2,4-Cyclopentane-trione, 3-(2- pentenyl).

TABLE 2: PHYTOCHEMICALS REPORTED IN GC-MS ANALYSIS OF METHANOLIC LEAF EXTRACT OF *T. FALCIFORMIS*

S. no.	R.T	Peak Area%	Compound name	Mol. Wt	Mol. formula
1	9.537	0.27	Benzoic acid, methyl ester	136	$C_8H_8O_2$
2	10.694	0.43	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144	$C_6H_8O_4$
3	11.618	2.31	Naphthalene	128	$C_{10}H_8$
4	12.445	0.33	Benzofuran, 2,3-dihydro-	120	C_8H_8O
5	14.209	0.49	2-methoxy-4-vinylphenol	150	$C_9H_{10}O_2$
6	18.004	0.18	2-Hydroxy-1-(1'-pyrrolidiyl)-1-buten-3-one	155	$C_8H_{13}NO_2$
7	18.335	0.25	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	180	$C_{11}H_{16}O_2$
8	18.804	0.18	1,2,4-Cyclopentanetrione, 3-(2-pentenyl)-	180	$C_{10}H_{12}O_3$
9	19.217	0.27	2h-1-benzopyran, 7-methoxy-2,2-dimethyl-	190	$C_{12}H_{14}O_2$
10	19.308	0.21	1-methyl-6-(3-methyl-buta-1,3-dienyl)-7-oxa-bicyclo[4.1.0]heptane	178	$C_{12}H_{18}O$
11	20.428	0.39	4,4,5,8-Tetramethylchroman-2-olg	206	$C_{13}H_{18}O_2$
12	20.865	0.41	2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethyl-	220	$C_{13}H_{16}O_3$
13	22.329	0.20	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	196	$C_{11}H_{16}O_3$
14	23.167	0.69	Neophytadiene	278	$C_{20}H_{38}$
15	23.241	0.35	2-Pentadecanone, 6,10,14-trimethyl-	268	$C_{18}H_{36}O$
16	23.525	0.23	Phthalic acid, isobutyl trans-dec-3-enyl ester	360	$C_{22}H_{32}O_4$
17	23.799	0.28	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	$C_{20}H_{40}O$
18	24.445	0.29	Hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$
19	25.101	13.89	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$
20	26.097	0.45	Palmitic Acid, TMS derivative	328	$C_{19}H_{40}O_2Si$
21	26.375	0.22	Heptadecanoic acid	270	$C_{17}H_{34}O_2$
22	26.832	0.79	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	292	$C_{19}H_{32}O_2$
23	27.031	1.07	Phytol	296	$C_{20}H_{40}O$

24	27.183	0.25	Octadecanoic acid, methyl ester	298	$C_{19}H_{38}O_2$
25	27.525	14.34	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	278	$C_{18}H_{30}O_2$
26	27.780	3.28	Octadecanoic acid	284	$C_{18}H_{36}O_2$
27	28.313	0.29	.alpha.-Linolenic acid, TMS derivative	350	$C_{12}H_{38}O_2Si$
28	30.165	0.86	1-Phthalimidoacetyl-2-carboethoxyhydrazine	291	$C_{13}H_{13}N_3O_5$
29	32.559	23.86	6-(2-ethoxyphenyl)-5-nitro-2-piperidinone	264	$C_{13}H_{16}N_2O_4$
30	33.407	1.13	2,2-dimethyl-7-hydroxy-6-[(2'-phenylethenyl)carbonyl]-(benzo-2h-pyran)	306	$C_{20}H_{18}O_3$
31	33.939	10.04	2-Phenyl-4-anilino-6[1H]-pyrimidinone	263	$C_{16}H_{13}N_3O$
32	35.219	0.82	8-(2,3-Dihydroxy-3-methylbutyl)-7-methoxy-2H-chromen-2-one	278	$C_{15}H_{18}O_5$
33	35.702	1.31	2-(3-methoxy-5-methyl-benzyliden)-5,7-dimethoxy-indan-1-on	324	$C_{20}H_{20}O_4$
34	36.401	1.29	3,6-heptanooxepin-4,5-dicarbonsaure-dimethylester	306	$C_{17}H_{22}O_5$
35	36.571	0.63	1H-Pyrido[3,4-b]indole, 2,3,4,9-tetrahydro-6-methoxy-1-methyl-	216	$C_{13}H_{16}N_2O$
36	36.792	0.71	2-benzoyl-3-methyl-4,8-dimethoxybenzo[1,2-b:5,4-b]difuran	336	$C_{20}H_{16}O_5$
37	37.166	1.14	[1]benzothieno[2,3-c]naphtho[1,2-g]quinoline	335	$C_{23}H_{13}NS$
38	37.383	0.34	2-Methylthio-4-oxo-4H-quinolizine-3-carboxamide	234	$C_{11}H_{10}N_2O_2S$
39	37.747	0.39	2H-1-benzopyran-6-ol, 3,4-dihydro-2,2-dimethyl-4-(1-methylethyl)-7-octyl-	332	$C_{22}H_{36}O_2$
40	38.199	0.84	Glycine, N-(4-butylbenzoyl)-, hexyl ester	319	$C_{19}H_{29}NO_3$
41	38.359	0.88	1h-isoindole-1,3(2h)-dione, 2,2'-(1,3-propanediyl)bis-	334	$C_{19}H_{14}N_2O_4$
42	38.543	0.81	pyrrolidine, 1,1'-(5-methyl-1,3-phenylene)bis-	230	$C_{15}H_{22}N_2$
43	38.745	0.30	4h,8h-benzo(1,2-b:3,4-b')dipyran-4-one, 2,3-dihydro-5-methoxy-8,8-dimethyl-2-phenyl-(s)-	336	$C_{21}H_{20}O_4$
44	38.906	0.54	Disiloxane, hexaethyl-	246	$C_{12}H_{30}OSi_2$
45	39.440	0.75	Disiloxane, hexaethyl-	246	$C_{12}H_{30}OSi_2$
46	40.010	0.21	6-benzyl-2,5-dimethyl-3-phenylpyrazolo[1,5-a]pyrimidin-7-ol	329	$C_{21}H_{19}N3O$
47	40.927	0.71	5-[2-(3-allyl-2-hydroxybenzylidene)hydrazino]-3-(methylsulfanyl)-4-isothiazolecarbonitrile	330	$C_{15}H_{14}N_4OS_2$
48	44.500	1.48	1h,3h-furo[3,4-c]furan, 1,4-bis(3,4-dimethoxyphenyl)tetrahydro-,[1r-(1.alpha.,3a.alpha.,4.alpha.,6a.alpha.)]	386	$C_{22}H_{26}O_6$
49	44.660	1.68	Stigmasterol	412	$C_{29}H_{48}O$
50	46.227	3.18	.gamma.-Sitosterol	414	$C_{29}H_{50}O$
51	48.635	0.96	24-Norursa-3,12-diene	394	$C_{29}H_{46}$
52	50.340	0.60	2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-3,4-dihydro-2h-chromen-6-yl hexofuranoside	592	$C_{35}H_{60}O_7$
53	55.251	2.21	Phytyl tetradecanoate	506	$C_{34}H_{66}O_2$

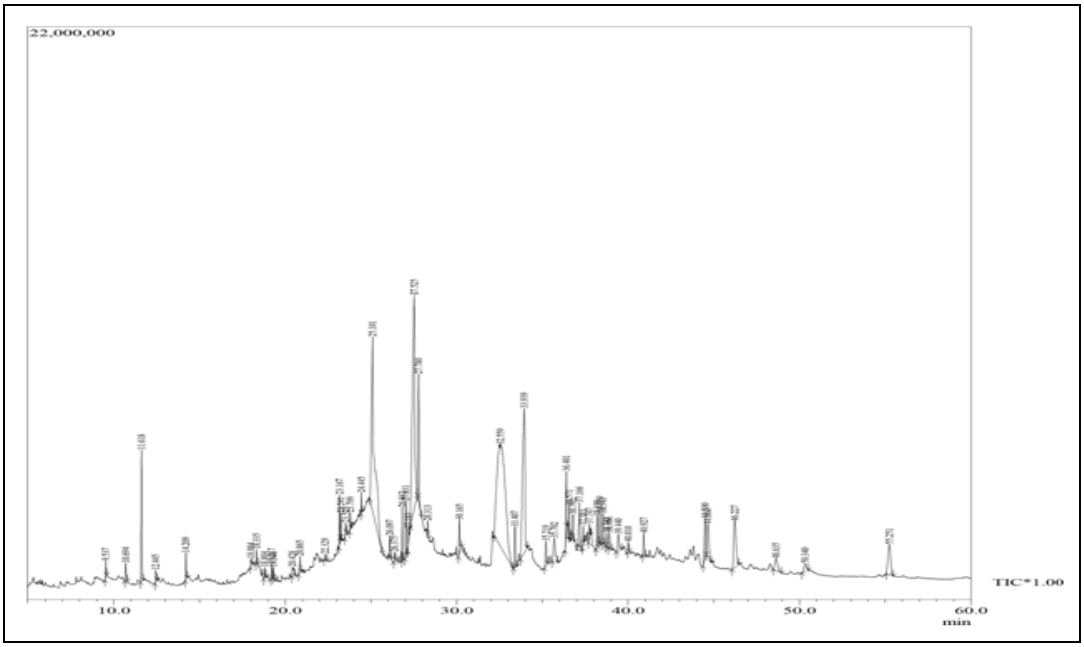
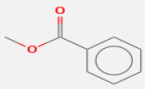
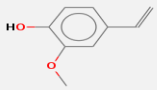
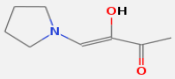
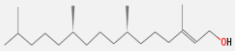
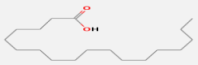
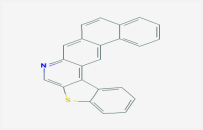
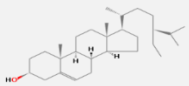
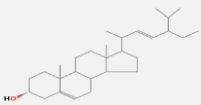


FIG. 2: CHROMATOGRAM OF METHANOLIC EXTRACT OF *T. FALCIFORMIS*

TABLE 3: BIOACTIVITY OF SOME COMPOUND REPORTED IN METHANOLIC EXTRACT OF LEAF OF *T. FALCIFORMIS*

S. no.	Compound name	Chemical group	Molecular structure	Biological activity
1	Benzoic acid, methyl ester	Methyl Ester		Antimicrobial properties [34]
2	Benzofuran, 2,3-dihydro-	Essential oils		Antifungal activity[32], Antioxidant activity[35]
3	Naphthalene	Aromatic hydrocarbon		Antimicrobial [36]
4	2-Methoxy-4-vinylphenol	Phenols		Anticancer [37]
5	2-Hydroxy-1-(1'-pyrrolidiyl)-1-buten-3-one	Alcohol		Antiseptic, Anesthetic, allergic dermatitis [38]
6	Neophytadiene	Sesquiterpene		Antimicrobial [39], Anti-inflammatory [31]
7	n-hexadecanoic acid	Fatty acid		Anti-inflammatory [40]
8	Palmitic acid, TMS derivative	Fatty acid derivative		Antitumor [41]
9	Phytol	Diterpene alcohol		anticancer, antioxidant, antitumor, antimicrobial [23]
10	Hexadecanoic acid, methyl ester	Fatty acid derivative		Anticancer [42]
11	Octadecanoic acid	Fatty acids		Antimicrobial [28]
12	[1]benzothieno[2,3-c]naphtho[1,2-g]quinoline	Alkaloid		Antinociceptive [16], antifungal activity [17, 43]. Antimalarial, antibacterial, anthelmintic, cardiotonic, anticonvulsant, anti-inflammatory and analgesic activity [43]
13	gamma.- Sitosterol	Sterols		Anticancer [44] Antidiabetic [45]
14	Stigmasterol	Sterols		Anti-inflammatory [25,46]

DISCUSSION: Bioactive compounds are responsible for the medicinal characteristics of the plants [15]. The presence of many significant secondary metabolites shows the potential of this plant for various therapeutical and pharmaceutical applications. The present analysis reveals that leaf contains terpene compounds in abundance along with alkaloids. Alkaloids are generally toxic to man and many of them have shown physiological activities; hence they are widely used in medicine [13]. Quinolene alkaloid also reported in leaf extract of *T. falciformis*, previous studies on Quinolene reveals that it has antinociceptive [16] and antifungal activity [17]. Likewise, terpenoids are isoprene molecules that are significant not only in plant growth and ecology but also provide a shield against insects.

These ranges from volatile (mono and sesquiterpenes), less volatile (diterpenes), and involatile compounds (triterpenoids steroids and carotenoid). Plant-based terpenoids are used in food, chemical industries, and pharmaceuticals and also used in the development of biofuel product [18]. Several studies reported the antimicrobial [19, 20], antioxidant, anti-inflammatory, antiparasitic, anticarcinogenic [21], antimalarial activity [22] of sesquiterpenes. Diterpenes Phytol reported in leaf extract, which shows anticancer, antioxidant, anti-inflammatory, diuretic, antitumor, chemopreventive, antimicrobial properties and also use in vaccine formation [23]. Other reported bioactive compound phytosterols are biogenetic precursors of many hormones and oviposition stimulants of some insects [13]. Additionally, phytosterols show lipid-lowering, anticancer, anti-inflammatory, and anti-allergy effects [24]. Stigmasterol is useful in the treatment of asthma [25].

Many studies have reported the advantages of phenolic compounds like antiaging, antioxidant, anti-inflammatory, anti-proliferative agents [26]. The fatty acids are active metabolites, and responsible for various medicinal properties, like hexadecanoic acid shows antibacterial and antifungal activity [27] and octadecanoic acid is antimicrobial in nature [28] palmitic acid and linolenic acid are known for their antibacterial and antifungal activity [29, 30]. The essential oils are found to be rich in sesquiterpenes and responsible for anti-inflammatory [31], antifungal [32,] and anticancer activity [33].

CONCLUSION: The GC-MS analysis of leaf extract of *T. falciformis*, which is an underexplored plant from the medicinal point of view, revealed the presence of 53 bioactive compounds. The medicinal utility of some of the compounds are already discussed which can justify the use of this plant for pharmaceutical preparations; however, the isolation of individual compound and study of its biological potential against various microorganisms is imminent for innovation of effective and safe medicines for treatment.

ACKNOWLEDGEMENT: Authors are thankful to the University grant commission, New Delhi, for providing financial assistance in the form of CAS program in the Department of Botany, Jai Narain Vyas University, Jodhpur, Rajasthan.

CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

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How to cite this article:
Vandana and Deora GS: Preliminary phytochemical screening and GC-MS analysis of methanolic leaf extract of *Tephrosia falciformis* Ramaswami from Indian Thar Desert. Int J Pharm Sci & Res 2020; 11(6): 3040-46. doi: 10.13040/IJPSR.0975-8232.11(6).3040-46.