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EVALUATION OF DIURETIC ACTIVITY OF CRUDE EXTRACTS OF LEAVES OF *FILICIUM DECIPIENS* AND ANALYSIS OF BIOMOLECULES PRESENT IN FRACTION OF METHANOLIC EXTRACT USING GC-MS TECHNIQUE

A. I. Basarikatti, V. Uppar and B. Padmashali *

Department of Chemistry, Rani Channamma University, Belagavi - 591156, Karnataka, India.

Keywords:

Bioactive chemical constituents, Diuretic activity, *Filicium decipiens*, GC-MS analysis and methanolic extract

Correspondence to Author:
Prof. Basavaraj Padmashali

Professor,
Department of
Chemistry Rani Channamma
University, Belagavi - 591156,
Karnataka, India.

E-mail: basavarajpadmashali@yahoo.com

ABSTRACT: *Filicium decipiens* belongs to the Sapindaceae family, which is commonly known as fern tree, is found in the Western Ghats of southern India, small highland areas of East Africa and Sri Lanka and it is a medium to a large evergreen tree. It is commonly cultivated in gardens and roadsides as ornamental, noise barriers and windbreak plant. *Filicium decipiens* traditionally used for the treatment of diabetes in India. The leaves of *Filicium decipiens* have been collected from the Western Ghats of southern India, shade dried and powdered well. The finely powdered leaves have been extracted with petroleum ether, chloroform, methanol and water successively with an increase in polarity. The methanolic extract was column chromate-graphed using silica gel G 100-200 mesh to get brown color crystalline solid, which was analyzed for the presence of bioactive chemical constituents using Gas chromatography-mass spectrometry (GC-MS) technique. GC-MS analysis revealed the presence of thirty chemical constituents. The four different crude extracts (petroleum ether, chloroform, methanol and water) of leaves of *Filicium decipiens* have been tested with a diuretic activity using the Lipschitz method. The methanolic extract exhibits diuretic activity.

INTRODUCTION: Medicinal plants are the "backbone" of traditional medicines. Plants have been used for medicinal purposes long before the prehistoric periods. India has been known for the rich repository of medicinal plants. The use of medicinal plants has always guided the look for new cures throughout the world for thousands of years and continues to endow with new remedies to humankind [1].

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The World Health Organisation (WHO) has projected that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy occupies the use of plant extracts and their bioactive components [2]. *Filicium decipiens* belongs to the Sapindaceae family. It is a large tree up to 30 m tall.

It is found in evergreen and semi-evergreen forests, which is native to Sri Lanka, the Western Ghats of southern India, and small highland areas of East Africa. *Filicium decipiens* is traditionally used as anti-diabetic agent in India and Sri Lanka [3]. It also showed a variety of biological activities, such as anti-fungal, anti-bacterial, anti-inflammatory, anti-oxidant and mollusicicidal activities [4, 5]. The chemical constituents present in plant such as

triterpenoidal saponins, norneohopane caffeate, sitosterol and flavonol glycosides [6]. The four new saponins have been isolated from the stem bark of *Filicium decipiens* [7]. But no report is available related to chemical constituents present in fraction of methanolic extract of leaves of *Filicium decipiens*. The aim of the present work is to investigate the bioactive chemical constituents present in a fraction of methanolic extract of leaves of *Filicium decipiens* using GC-MS technique and evaluation of the diuretic activity of four different (petroleum ether, chloroform, methanol and water) crude extracts of leaves of *Filicium decipiens*.

GC-MS is an analytical method that helps in the identification of chemical constituents present in the test material. It is a very powerful and sensitive instrument used to study trace amounts of chemicals of volatile material. The GC-MS can detect chemicals in amounts as small as pictograms [8, 9].

MATERIALS AND METHODS:

Collection of Plant Material: *Filicium decipiens* (Sapindaceae) leaves were collected from the campus of Sahyadri Science College, Shimoga (Karnataka). It was identified and authenticated from the ICMR-National Institute of Traditional Medicine Belagavi (Karnataka). The plant specimen has been preserved in the herbarium (RMRC-1388).

Preparation of the Extracts: The collected leaves were shade dried at room temperature (32 °C ± 2 °C), and it is made into a coarse powder. The powdered material (150 g) was subjected to Soxhlet extraction (48 h) with petroleum ether, chloroform, methanol and water (1.5 liters) successively. The methanolic extract is rich in plant constituents as indicated by phytochemical investigation and has shown better anti-oxidant effect [10, 11]. The methanolic extract was used for the separation of phytochemicals by a chromatographic method.

Isolation Method with Column Separation: Methanol extract (10 g) was subjected to column chromatography on silica gel (100-200) mesh and eluted with chloroform, ethyl acetate, and methanol with increasing polarity. Methanolic extract gave a brown color crystalline compound weighing 400 mg, which was named as AP1.

GC-MS Analysis: GC-MS analysis of the fraction of methanolic extract *i.e.,* AP1, was performed using a Perkin Elmer Clarus SQ8C instrument. DB-5 MS capillary standard non-polar column of length 30 meters was used. He was used as carrier gas at a flow rate of 1ml/min. Injection of the sample was 1 microlitre and injection temperature 250 °C, ion sources temperature 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min) with an increase of 10 °C /min to 200 °C /min then 5 °C /min to 280 °C /min ending with a 9 min, isothermal at 280 °C. The mass spectrum was taken at 70 eV, a scan-interval of 0.5 sec, and fragments from 40to 550 Da.

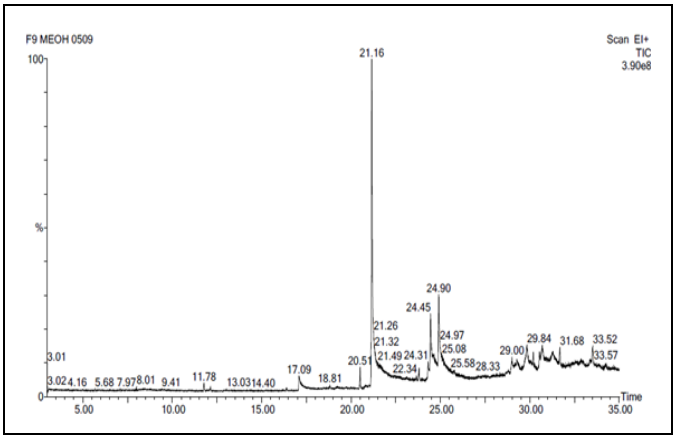


FIG. 1: GC-MS ANALYSIS OF FRACTION OF METHANOLIC EXTRACT OF LEAVES OF *FILICIUM DECIPIENS* I.E. AP1

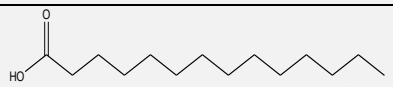
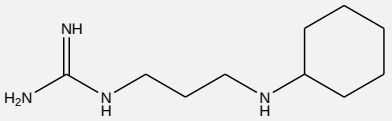
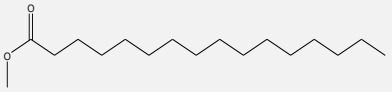
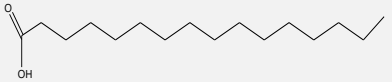
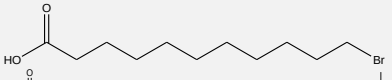
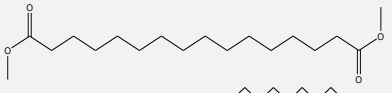
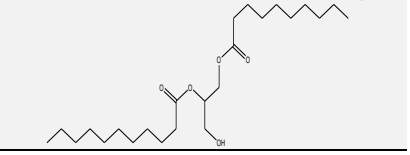
Diuretic Activity: Diuretics are a class of drugs that increases the rate of urine flow; however, clinically useful diuretics also increase the rate of excretion of Na^+ and an accompanying anion, usually Cl-. Sodium chloride in the body is the major determinant of extracellular fluid volume, and most clinical applications of diuretics are directed towards reducing the extracellular fluid volume by decreasing the total sodium chloride content of the body. Diuretics are used to treat heart failure, liver cirrhosis, hypertension, influenza, water poisoning, and certain kidney diseases [12].

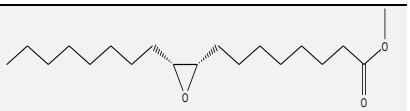
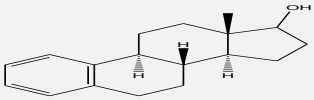
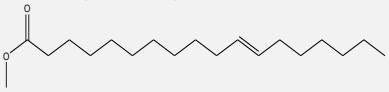
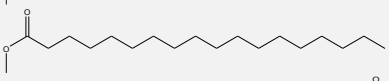
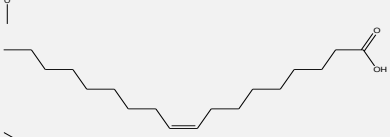
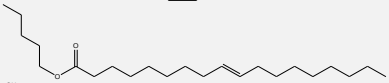
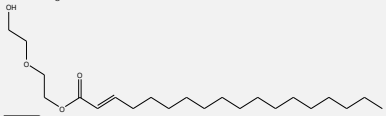
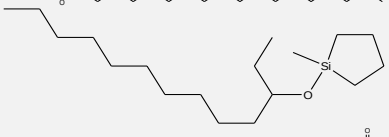
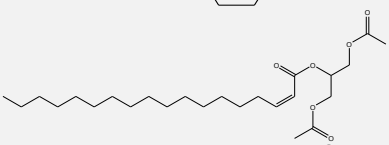
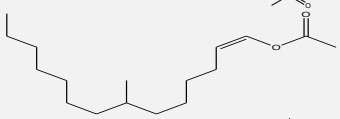
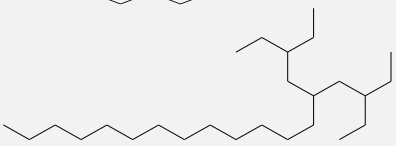
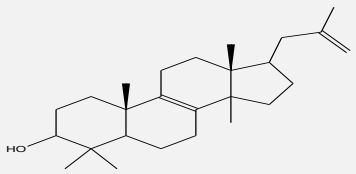
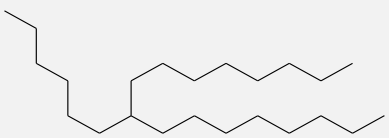
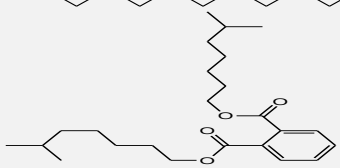
Wistar strain albino rats of either sex, weighing between 100-200 g. They were housed at 22 ± 2 °C with constant humidity 50–60% on the 12-h natural day and night cycles. They were fed with standard diet and water *ad libitum*. The experiments were carried out in accordance with the directions of the Institutional Animal Ethics Committee (IEC/2016/Ph.D. FDD/AB-03).

Furosemide was used as the standard drug. All the chemicals used in the present study were purchased from reliable sources and were of standard quality [13]. The diuretic activity was determined by following the procedure described by Lipschitz *et al.,* [14]. The selected animals were divided into 6 groups, each containing 4 animals. Group, I served as control and received 5 ml of 0.1% Tween-80 in distilled water orally. Group II received 40 mg/kg body weight of furosemide in 0.1% Tween-80 orally and served as standard. Group III, IV, V, and VI received the extracts at the dose of 600 mg/kg body weight suspended in 0.1% Tween-80 orally. Each group of animals was kept in different metabolic cages provided with a wire mesh at the bottom and a funnel to collect urine. Sieves made up of stainless steel were placed on the funnel to retain feces. Food and water were withdrawn 24 h prior to the experiment. Urine excretion was collected after 5 h. The results are tabulated in **Table 2**.

RESULTS AND DISCUSSION: GC-MS study of the fraction of methanolic extract of leaves of *Filicium decipiens i.e.,* AP1, leads to the identification of thirty chemical constituents present in it. The identification of chemical constituents is based on a molecular formula, retention time, molecular weight, and area percentage. The results of the present study were tabulated in **Table 1**. The identification of thirty chemical components like tetradecanoic acid ($C_{14}H_{28}O_2$), 1-(3- (Cyclohexylamino) propyl) guanidine ($C_{10}H_{22}N_4$), hexadecanoic acid methyl ester ($C_{17}H_{34}O_2$), n-hexadecoic acid ($C_{16}H_{32}O_2$), 11 bromoundecanoic acid ($C_{11}H_{21}BrO_2$), hexa-decanedioic acid dimethyl ester ($C_{18}H_{34}O_4$), hexadecanoic acid 1- (hydroxymethyl)-1, 2-ethanediyl ester ($C_{35}H_{68}O_5$), oxiraneoctanoic acid 3- octyl- methyl ester ($C_{19}H_{36}O_3$), estra-1, 3, 5(10)-trien-17-ol ($C_{18}H_{24}O$), 11-octadecenoic acid methyl ester ($C_{19}H_{36}O_2$), methyl stearate ($C_{19}H_{38}O_2$), oleic acid ($C_{18}H_{34}O_2$), trans 9-octadecenoic acid, pentyl ester ($C_{23}H_{44}O_2$), octadecenoic acid 2-(2-hydroxy-ethoxy) ethyl ester ($C_{22}H_{42}O4$), 1-methyl-1-(3-tridecyl) oxy-1- silacyclopentane ($C_{18}H_{38}OSi$), octadecenoic acid (Z)-2-(acetyloxy)-1-((acetyloxy) methyl) ethyl ester ($C_{25}H_{44}O_6$), 7-methyl-Z-tetradecen-1-ol acetate ($C_{17}H_{32}O_2$), octadecane 3-ethyl-5-(2-ethylbutyl) ($C_{26}H_{54}$), 8-Androsten-3-ol, 17-(2-methylallyl)-4, 4, 14-trimethyl ($C_{28}H_{44}O_2$), tetratetracontane ($C_{44}H_{90}$), heptadecane 9-hexyl ($C_{23}H_{48}$), diisooctyl phthalate ($C_{24}H_{38}O_4$), 17-pentatriacontene ($C_{35}H_70$), acetyl betulinaldehyde ($C_{32}H_{50}O_3$), androsterone acetate ($C_{21}H_{32}O_3$), 9-hexadecenoic acid 9-octadecenyl ester ($C_{34}H_{64}O_2$), beta-sitosterol ($C_{29}H_{50}O$), n-Butyl ricinoleate ($C_{22}H_{42}O$), heneicosane ($C_{21}H_{44}$) and heptadecane 9-octyl ($C_{25}H_{52}$).

TABLE 1: GC-MS ANALYSIS OF THE FRACTION OF METHANOLIC EXTRACT OF *FILICIUM DECIPIENS* I.E. AP1

S. no.	RT	Molecular formula	Molecular weight [g/mol]	peak area %	Name of the compound	Structure
1	17.08	$C_{14}H_{28}O_2$	228.37	1.06	Tetradecanoic acid	
2	17.22	$C_{10}H_{22}N_4$	198.31	0.61	1-(3-(Cyclohexylamino)propyl)guanidine	
3	20.51	$C_{17}H_{34}O_2$	270.45	0.63	Hexadecanoic acid, methyl ester	
4	21.16	$C_{16}H_{32}O_2$	256.4	2.68	n- Hexadecanoic acid	
5.	22.10	$C_{11}H_{21}BrO_2$	265.19	0.65	11 bromoundecanoic acid	
6.	22.16	$C_{18}H_{34}O_4$	314.46	0.47	Hexadecanedioic acid, dimethyl ester	
7	22.25	$C_{35}H_{68}O_5$	568.92	0.39	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	

8	22.43	$C_{19}H_{36}O_3$	312.49	0.91	Oxiraneoctanoic acid, 3- octyl-, methyl ester, cis-	
9	22.57	$C_{18}H_{24}O$	256.38	0.65	Estra-1,3,5(10)-trien-17-ol	
10.	23.81	$C_{19}H_{36}O_2$	296.49	0.49	11-Octadecenoic acid methyl ester	
11	24.31	$C_{19}H_{38}O_2$	298.50	0.77	Methyl stearate	
12	24.44	$C_{18}H_{34}O_2$	282.46	3.92	Oleic acid	
13	24.64	$C_{23}H_{44}O_2$	352.60	0.90	trans 9-Octadecenoic acid, pentyl ester	
14	24.90	$C_{22}H_{42}O_4$	370.57	4.33	Octadecenoic acid,2-(2-hydroxyethoxy) ethyl ester	
15	28.80	$C_{18}H_{38}OSi$	298.58	0.44	1-Methyl-1-(3-tridecyl)oxy-1-silacyclopentane	
16	28.99	$C_{25}H_{44}O_6$	440.62	1.35	Octadecenoic acid (Z)-, 2-(acetyloxy)-1-((acetyloxy)methyl)ethyl ester	
17	29.15	$C_{17}H_{32}O_2$	268.44	0.51	7-Methyl-Z-tetradecen-1-ol acetate	
18	29.26	$C_{26}H_{54}$	366.71	0.95	Octadecane, 3-ethyl-5-(2-ethylbutyl)	
19	29.35	$C_{28}H_{44}O_2$	412.64	0.53	8-Androsten-3-ol, 17-(2-methylallyl)-4,4,14-trimethyl	
20	29.83	$C_{44}H_{90}$	619.20	3.54	Tetratetracontane	
21	30.19	$C_{23}H_{48}$	324.63	0.64	Heptadecane, 9-hexyl	
22	30.54	$C_{24}H_{38}O_4$	390.56	0.81	Diisooctyl phthalate	
23	30.69	$C_{35}H_{70}$	490.94	2.96	17-Pentatriacontene	

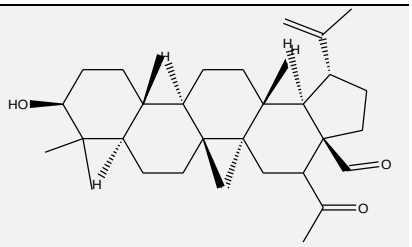
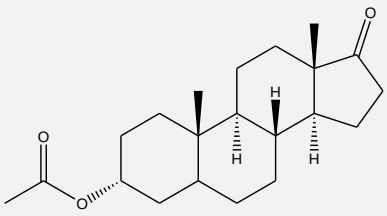
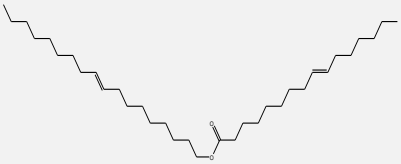
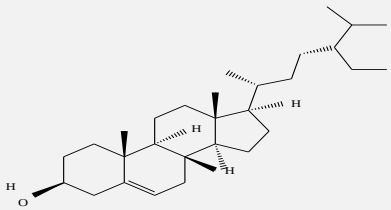
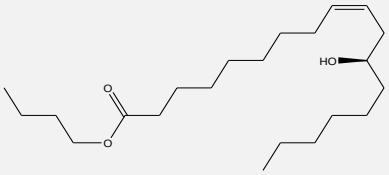
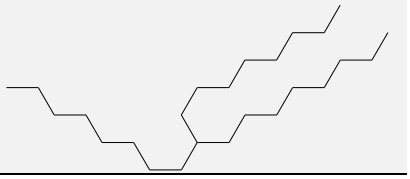
24	30.87	$C_{32}H_{50}O_3$	482.74	0.50	Acetyl betulinaldehyde	
25	30.92	$C_{21}H_{32}O_3$	332.48	0.63	Androsterone acetate	
26	31.02	$C_{34}H_{64}O_2$	504.88	0.68	9-hexadecenoic acid, 9-octadecenyl ester	
27	31.29	$C_{29}H_{50}O$	414.70	2.23	Beta-sitosterol	
28	31.41	$C_{22}H_{42}O_3$	354.57	0.50	n-Butyl ricinoleate	
29.	31.68	$C_{21}H_{44}$	296.58	0.90	Heneicosane	
30	33.52	$C_{25}H_{52}$	352.69	1.19	Heptadecane 9-octyl	

TABLE 2: DIURETIC ACTIVITY OF THE EXTRACTS OF *FILICIUM DECIPIENS*

Group	Compound	Dose (mg/kg)	Volume of urine (ml) collected after 5 h	T/S (Lipschitz values)
I	Control	600	08	0.27
II	Standard	600	29	1.00
III	Petroleum ether extract	600	14	0.45
IV	Chloroform extract	600	17	0.54
V	methanol extract	600	12	0.68
VI	Water extract	600	8	0.41

CONCLUSION: The Lipschitz value for the standard drug Furosemide is taken as 1. The tested extracts of *Filicium decipiens* showed less T/S value compared to standard. Hence, the only methanolic extract exhibited diuretic property, and the other extracts showed less diuretic activity. The methanolic extract was column chromate-graphed using silica gel G 100-200 mesh to get brown color crystalline solid, which was analyzed for the presence of bioactive chemical constituents using Gas chromatography-mass spectrometry (GC-MS) technique.

GC-MS analysis revealed the presence of thirty chemical constituents. The identification of these compounds in the plant serves as the basis in determining the possible biological activities.

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CONFLICTS OF INTEREST: Nil

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