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Isolation of active components derived from *Teramnus labialis.*

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ABSTRACT

The objective of the present investigation was to isolate the active components present in whole plant of .. The plant were extracted with various solvents (pet. ether, ethyl acetate and methanol), methanol was found to be more active among them. The preliminary phytochemical results revealed that flavonoids and amino acids as active constituents in methanolic extract of *Teramnus labialis* .The methanolic extract of *Teramnus labialis* was undergone column chromatography with different solvent fractions. Hence, two compounds were isolated from methanolic extract of *Teramnus labialis* with the compound 1 was eluted with Fraction 8-11 (eluted petroleum ether – Benzene 70:30), gave a solid designated as compound 1 (100 mg) and fraction 40 – 43 (eluted Benzene – ethyl acetate 10:90, gave a solid designated as compound 2 (150 mg) and fraction 62-67 (eluted ethyl acetate – methanol 90:10) gave a solid designated as compound 3 (162 mg). The structures of the two isolated compounds were characterized by using FT-IR, NMR and Mass spectrophotometric methods. Thus, the compound 1 was characterized as 5, 6 Dihydro – 6 dodekylpyran – 2 one and its molecular formula is deduced as C17 H28 O2; Structure of compound 2 is proposed as to 4' 5, 6' – Trihydroxy – 7-methoxy-isoflavanone – 2 carboxylic acid and its molecular formula is deduced as C17 H12 O8.Therefore, further biological investigations need to be carried out isolated compounds present in this plant.

Keywords: Teramnus labialis, Column chromatography, FT-IR, MASS,NMR Spectram

INTRODUCTION

Teramnus labialis (L) spreng (Family; Fabaceae) is a herb, commonly known as mashaparni and a well known medicinal plant in the Ayurvedic system of medicine. It has been reported to be useful in treating rheumatism, tuberculosis, nerve disoders, paralysis and catarrhs [1-3], and chemical analysis and nutritional assessment [4]. The plant used as antihyperglycemic activity [5], anti-inflammatory activities [6], anoval bioactive flavonol glycoside from teramnuslabialis [7]. Therefore, the objective of the present investigation was to isolation of active components derived from whole plant of *Teramnus labialis* by using FT-IR, NMR and mass spectrophotometric methods

MATERIALS AND METHODS

Plant material

The tuberous root of *Teramnus labialis* (Linn), were collected from Kilikulam, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The tuberous root of *Teramnus labialis* (Linn)were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

PREPARATION OF EXTRACTS

The above powdered materials were successively extracted with methanol (60-800C) by hot continuous percolation method in Soxhlet apparatus [8]for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained. The methanolic extract was stored in screwcap vial at 40C until further use.

Preliminary phytochemical screening

The extract was subjected to preliminary phytochemical screening for the detection of various plant constituents present. The various extracts of *Teramnus labialis* was subjected to the following chemical tests such as tests for Alkaloids [9, 10], test for Carbohydrates[9], tests of Glycosides ,testsforPhytosterol[10], test for Coumarins , test for Flavonoids [11,12], test for Tannins and Phenolic compounds [13], tests for Proteinsand Amino Acids [6], test for Saponins, testfor Fixed Oils [9]

Preliminary phytochemical screening

The methanolic extract of *Teramnus labialis* was screened for its phytochemical constituents. The phytochemical screening results are shown Table 1.

S.No.	**Name** of **Test**	***Teramnus labialis***
1.	Test for Alkaloids	+
2.	Test of Carbohydrates	+
3.	Test for Glycosides	+
4.	Test for Phytosterol	+
5.	Test for fixed oils and fats	+
6.	Test for saponins	_
7.	Test for tannins and phenolic compounds	+
8.	Test for Proteins and Free Amino Acids	-
9.	Test for Flavonoids	+
10.	Test for Liginin	_
11.	Test for Terpenes	_
	Where + =Positive	**– = Negative**

TLC Chromatographic Profiles

The methanolic extract of of*Teramnus labialis* was dissolved in the mother solvent and spotted on plates 2 c.m above its bottom. Most of the sample for application were between o.1-1%. The applied spots were spots were of equal size as far as possible and diameter ranging from 2-3m.m.

The mobile phase(solvent system) for TLC was developed by trial and error using solvents which were differing in polarities as given below in Table.2

Table 2.TLC analysis of methanolic extract of *Teramnus labialis*

S. No.	Solvent system	No. of spots	Rf value
1.	Benzene:Ethylacetate:Methanol 70:20:10	04	0.17 0.28 0.39 0.44

Isolation of compounds by column chromatography

The methanol extract of *Teramnus labialis* was subjected to column chromatographic separation using normal phase silica gel column. The brown solid (15 g methanolic extract of*Teramnus labialis* was adsorbed on silica gel (15 g) and transferred to a column of silica gel (200g equilibrated with petroleum ether). Elution was performed with petroleum ether (100%), petroleum ether: benzene (80:20), petroleum ether: benzene (60:20), petroleum ether: benzene (40:60), petroleum ether: benzene (20:80), benzene (100), benzene : ethyl acetate(90:10), benzene : ethyl acetate (80:20), benzene: ethyl acetate (60:40), benzene: ethyl acetate(40:60), benzene: ethyl acetate(20:80),ethyl acetate (100%), methanol(100%) ethyl acetate: methanol (80:20) ethyl acetate: methanol (60:40) , ethyl acetate: methanol (40:60), ethyl acetate: methanol (20:80), ethyl acetate: methanol (70:30), ethyl acetate: methanol (60:40)and methanol(100). Fractions of 100ml were collected every time, distilled off the solvent and the homogeneity of the resulting residues was examined on TLC by using different solvent systems and similar fractions, identified by their TLC behaviour were mixed together.

Fraction 8-11 (eluted petroleum ether – Benzene 70:30), gave a solid designated as compound 1 (100 mg) and fraction 40 – 43 (eluted Benzene – ethyl acetate 10:90, gave a solid designated as compound 2 (150 mg) and fraction 62-67 (eluted ethyl acetate – methanol 90:10) gave a solid designated as compound 3 (162 mg).

Structure and elucidation of isolated compounds:

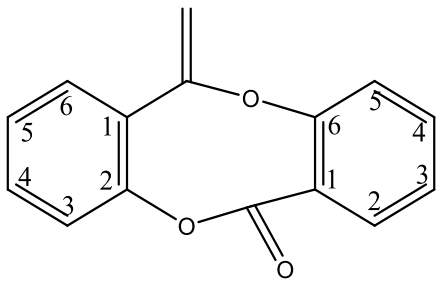
The isolated compounds were analyzed by FT-TR, ^{1}HNMR &^{13}CNMR and Mass Spectrum. As per the spectral analysis the structure of compound-1 is proposed as 12-methylenedibenzo [b, f] [1, 5] dioxocin – 6 (12-H) one and its molecular formula is deduced as $C_{15}H_{10}O_3$;

Structure of compound 1 is proposed as 5, 6 Dihydro – 6 dodekylpyran – 2 one and its molecular formula is deduced as $C_{17}H_{28}O_2$;

Structure of compound 2 is proposed as to 4’ 5, 6’ – Trihydroxy – 7-methoxy- isoflavanone – 2 carboxylic acid and its molecular formula is deduced as $C_{17}H_{12}O_8$.

COMPOUND 1

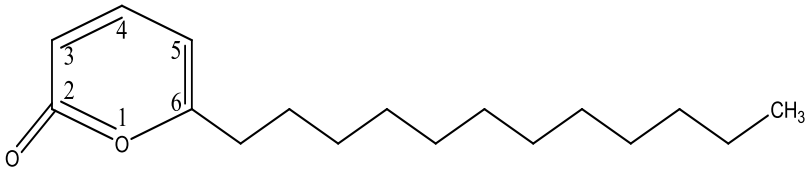
IR (1KBr)3071-3012 (γC-H aromatic), 1686 (γC=0), 1602 (γC=Caromatic) and 1290 (γC-O) am. **^{1}HNMR:**(DMSO-d_6, 400MH$_Z$) : δ 8.20 – 8.19 (d, 2H, C_6-H & C_2-H), 8.14-8.12 (d, 2H, C_3-H & C_5-H) and 7.96-7.37 (m, 4H, $C_{4,5,4',3'}$ – H), ppm. **^{13}CNMR :**(DMSo-d_6 400MH$_z$) : δ 167, 162, 150, 135, 134, 133, 132, 131, 130, 129, 128, 127, 124 and 122 ppm



Structure of compound-1 proposed 12-methylenedibenzo [b, f] [1, 5] dioxocin – 6 (12-H) one and its molecular formula is deduced as C_{15} H_{10} O_{3}.. Molecular formula: C_{15} H_{10} $O_{3,}$ Molecular weight :238

COMPOUND 2

IR (1KBr):2917 (γ_{C}-Haliphatic), 1735 (γC=0), 1473 (γC=0alkene) and 1173 (γC-0-C) cm^{-} ^{11}**HNMR:**($CDCl_3$, $400MH_Z$) : δ 5.33 – 5.32 (d, 1H, C_3-H)4.02-3.99, (t, 1H, C_4-H), 3.61-3.60 (d, 2H, C_5-H), 3.52-3.52 (t, 1H, C_6 –H) and 2.26-0.84 (m, 254, C_6-CH_2 $(CH_2)_{10}$ CH_3) ppm. ^{13}**CNMR:**($CDCl_3$-$400MH_z$) : 174, (C_2), 64 (C_3), 49 (C_4), 45 (C_6), 42 (C_5), 39, 36, 34, 31, 29, 28, 27, 25, 24, 22 and 19 (C_6 – $(C)_{12}$) ppm . **Structure Teramnus – 2**



Compound 2 was proposed to 5, 6 Dihydro – 6 dodekylPyran – 2 one
Molecular formula: C_{17} $H_{30}O_2$,Molecular weight : 266

CONCLUSION

From the above reports, three compounds were isolated from methanolic extract of *Teramnus labialis* (Linn.) such as compound-1 12-methylenedibenzo [b, f] [1, 5] dioxocin – 6 (12-H)(C_{15} H_{10} $O_{3)}$. compound 2 was characterized as 5, 6 Dihydro – 6 dodekylPyran – 2 one(C_{17} H_{30} $O_{2)}$in this plant. Furthermore, biological investigations are required for these isolated compounds.

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