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Phytoconstituents and biological screening of aqueous extracts of *Phyla nodiflora* Linn.

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ABSTRACT

The aqueous extracts of dried roots and leaf, stem and flower parts of *Phyla nodiflora* Linn were subject for the preliminary Phytoconstituents analysis and ascertained that the plant possesses Glycosides, alkaloids, carbohydrates, proteins and amino acids, flavanoids, saponins, phytosterols and Terpenoids. Analgesic activities of the aqueous plant extracts of *Phyla nodiflora* Linn were assessed using acetic acid developed writhing peripheral analgesic method and hot plate central analgesic method in the mice. Both of the extracts showed significant peripheral and central analgesic activity. But the aerial parts of the extracts have shown more analgesic activity than root of the plant. The crude plant extracts and their solvent soluble fractions were subjected to antibacterial evaluation against four different bacteria Escherichia coli, Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa. The result of anti bacterial screening revealed that aqueous extract failed to inhibit Escherichia coli, Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa.

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INTRODUCTION

Phyla nodiflora Linn. (Verbenaceae) is generally called as Bhujokra in Hindi, Ratoliya in Gujarati and Poduthalai in Tamil (C Seniya *et al.,* 2011, SG Alsabri *et al.,* 2013, M Raza et al, 2012, E Malakodi and A Manoharan 2013). It has been found at fairly high temperature parts of India ascending up to 900m in the hills. It is common in wet places, canal edges along irrigation channels and river banks (Mohamoud Rafieian Kopaei, 2012). It is a creeping, branched perennial herb with branches spreading generously and rooting at the nodes. The stem is woody at the base and light violet if exposed to the sun; it is straight, pale creamy or whitish brown in color and shriveled. The leaves are small, simple, obtuse and obovate, somehow deeply and sharply serrate towards the apex; both surfaces are shiny and hairy white strigose hairs. The root of this plant is tough, knotty with fibrous fracture and whitish wood (Sharma R A and Renu singh, 2013).

In literature review, it revealed that the aerial parts are used as diuretic, emmenogogue, parasiticide, febrifuge and cooling (C Sumitra *et al.,* 2013, KV Yogesh *et al.,* 2004). According to traditional uses, the plant is useful in cold and fevers, stomachic, used in lack of bowel movements and for pain in knee joints and in lithiasis (Mehreen Jabeen *et al.,* 2016, Evelyne Priya S and Ravindhran R, 2015).

Phyla nodiflora contains flavonoids, carbohydrates, sterols, essential oil, resin, non- glucosidal bitter substances, tannins and other constituents (Kavitha B *et al.,* 2012). Several workers have already reported many pharmacological properties including antispasmodic (Sujatha Dodoala *et al.,* 2010), hair afflictions (Hardy JD *et al.,* 1940), anti-inflammatory and antipyretic (Eddy NB *et al.,*

1953), anti Helicobacter pylori activity (Coe FG and Anderson GJ, 1996), hypotensive activity (Zaika LL, 2007), antinociceptive (Cowan MM, 1999) and antifungal (Malik HMA 2001). However, it was found that there were no reports on analgesic and antibacterial activities for the plant. Hence, the study was designed to verify the claims of traditional systems of medicine.

MATERIALS AND METHODS

Collection of the plant materials and extraction:

The plant materials used in this study consisted of the whole plant of *Phyla nodiflora,* collected in and around Namakkal & Kolli hills. The collected plant was authenticated by Dr.S.Senthil Kumar, Department of Botany, Vivekanandha College of Arts and Science, Elayampalayam, Tiruchengode. The shade dried whole plant was powdered mechanically and into coarse powder. About 1000g of aerial dried powder were extracted using 2500ml of water by using cold maceration method. About 200g of root dried powder were extracted using 800ml of water by using cold maceration method.

After the completion of the maceration, extracts were filtered and removed the solvent by evaporating them to dryness on a water bath. The obtained brown colour residue was stored in a dessicator.

Identification of Phytochemical Constituents

The extracts of both aerial and root parts of *phyla nodiflora Linn* were subjected for the preliminary Phytoconstituents screening for identifying the various active constituents present.

Analgesic Activity

The Analgesic activity of the aqueous plant extracts of *Phyla nodiflora* Linn were assessed by using acetic acid induced writhing peripheral analgesic method and hotplate central analgesic method in mice.

Hot plate method

Hot plate method was carried out as per method described by Eddy and Leimbach. The prescreened *Swiss* albino mice showing the reaction time of 3 to 5 sec and were selected and randomly divided to groups of four of having six mice per group. The group-1 was given with 1% CMC solution for the body weight of 10 ml/kg (control group), the group-2 was given with codeine 5 mg / kg, p.o. (Standard group), while the groups 3 and 4 were given 200, 400 mg/kg of *Phyla nodiflora* Linn. The animals were placed on the hot plate which was maintained at the temperature of 55±1°C. The reaction time for the control and treated animals were recorded until the animals had shown a licking or jumping movement. A time of 10 seconds was considered as cut off time. Thus, the reaction times at various intervals were recorded at 0, 30, 60, 90 and 120 min after the administration of the test drug.

Acetic acid induced writhing method

The acetic acid induced writhing model was used with slight modification. A twenty four albino mice of having both sex were randomly divided into six groups of having four mice per group. The group-1 was given with normal saline of 10 ml/kg (control group), the group-2 was given with the drug Diclofenac sodium of 20 mg /kg i.p. (Standard group), while groups-3 and 4 were given with the aerial parts of extract made with the two different concentrations i.e., a low dose of 200mg/kg and high dose 400 mg/kg of *Phyla nodiflora* Linn. And Group-5 and 6 were given with the root part of extract made with the two different concentrations i.e., a low dose of 200mg/kg and high dose 400mg/kg. All the above extracts of different concentrations were administered by gastric gavage. After an hour of administration of drug and extract, 0.1% acetic acid (10 ml/kg) was given intra peritoneally (i.p) to all the mice for inducing pain characterizing either abdominal constrictions or writhes. The number of writhes observed in each mouse were counted for the period of 10minutes and recorded. The percentage of protection against abdominal writhing was used to assess the degree of analgesia which was calculated by using the formula. The percentage of inhibition of writhing = (Wc – Wt/Wc) x 100 (Where, Wt represents the mean number of writhes in mice treated with test drug and Wc represents the mean number of writhes in control group).

RESULTS AND DISCUSSION

The plant *phyla nodiflora Linn.* Of family Verbenaceae was collected from the Namakkal and its surrounding areas in the month of January 2017. The plant material was dried in shade, after the process of drying aerial and root was separated. The size was reduced mechanically into a coarse powder. The dried coarse powder of *phyla nodiflora* linn root and aerial part of the plant was subjected to aqueous solvent extraction process by cold macer- ation method. Then all the extracts were subjectedto qualitative analysis.

The preliminary phytochemical investigation of *Phyla nodiflora Linn* showed the presence of carbohydrates, proteins and amino acids, flavanoids, glycosides, saponins, phytosterols, alkaloids and terpenoids in the plant extract. And the results of preliminary phytochemical analysis for the aqueous

extracts of *phyla nodiflora* linn are shown in Table 1.

The analgesic activity of *Phyla nodiflora* Linn. was tested upon adult mice by Hot plate method. The aqueous extracts of aerial and root of plant at low dose of 200mg/kg and high dose of 400mg/kg have shown significant analgesic activity when compared to the standard Morphine sulphate 5mg/kg. The results of analgesic activities of aqueous extracts of *phyla nodiflora* linn by hot plate method have shown in table.2.

The analgesic activity of *Phyla nodiflora* Linn. was tested upon adult mice by acetic acid induced abdominal writhing method. The aqueous extracts of

Table 1: Preliminary phytochemical investigation of *Phyla nodiflora* Linn

S.No.	Test	Aqueous Extract of aerial parts	Aqueous Extract of root parts
1.	Carbohydrates	+	+
2.	Proteins & Amino Acids	+	+
3.	Flavanoids	+	+
4.	Glycosides	+	+
5.	Saponins	+	+
6.	Phytosterols	+	+
7.	Tannins & Phenolic Compounds	-	-
8.	Alkaloids	+	+
9.	Gum & Mucilage	-	-
10.	Fixed oil & fats	-	-
11.	Terpenoids	+	+

+ indicates presence and - indicates absence

Table 2: Analgesic activity of *Phyla nodiflora* Linn by Hot plate method

ANIMAL GROUPS	TREATMENT DOSE mg/kg	No. of mice	BODY WEIGHT	0 MINS	30 MINS	60 MINS	90 MINS	120 MINS
Group – I	CONTROL: Normal saline	1	25gm	3sec	4sec	5sec	5sec	4sec
		2	26gm	1sec	3sec	4sec	4sec	2sec
		3	26gm	4sec	5sec	3sec	2sec	4sec
		4	28gm	3sec	3sec	2sec	1sec	2sec
MEAN				2.75	3.75	3.5	3.25	3
Group – II	STANDARD: Morphine sulphate 5mg/kg (I.P)	1	27gm	9sec	11sec	13sec	13sec	12sec
		2	26gm	13sec	14sec	14sec	12sec	11sec
		3	25gm	10sec	10sec	15sec	14sec	12sec
		4	26gm	5sec	8sec	11sec	8sec	7sec
MEAN				9.25	10.45	13.25	11.75	10.5
Group – III	*Phyla nodiflora* (Aerial) low dose 200mg/kg	1	27gm	9sec	10sec	13sec	13sec	13sec
		2	30gm	13sec	13sec	14sec	14sec	13sec
		3	29gm	9sec	9sec	10sec	12sec	9sec
		4	25gm	5sec	9sec	11sec	8sec	12sec
MEAN				9	10.25	12	11.75	11.75
Group – IV	*Phyla nodiflora* (Aerial) high dose 400mg/kg	1	28gm	10sec	11sec	12sec	14sec	13sec
		2	27gm	13sec	13sec	14sec	14sec	14sec
		3	29gm	9sec	13sec	11sec	12sec	11sec
		4	30gm	7sec	8sec	9sec	10sec	10sec
MEAN				9.7	11.25	11.5	10.5	12
Group - V	*Phyla nodiflora* (Root) low dose 200mg/kg	1	29gm	7sec	8sec	10sec	11sec	8sec
		2	27gm	11sec	12sec	11sec	13sec	12sec
		3	26gm	5sec	7sec	8sec	10sec	5sec
		4	27gm	3sec	4sec	4sec	6sec	3sec
MEAN				6.5	7.75	8.25	10	7
Group - VI	*Phyla nodiflora* (Root) high dose 400mg/kg	1	25gm	8sec	9sec	10sec	11sec	8sec
		2	28gm	12sec	12sec	13sec	13sec	12sec
		3	29gm	7sec	8sec	9sec	9sec	8sec
		4	26gm	4sec	4sec	5sec	6sec	5sec
MEAN				7.75	8.25	9.25	9.75	8.25

Table 3: Analgesic activity of Phyla nodiflora Linn by acetic acid induced abdominal writhing method

GROUPS	NO OF MICE	BODY WEIGHT	NO. OF WRITHING RESPONSE	% INHIBI-TION
Group – I (control) Normal saline	1.	26gm	46	0
	2.	28gm	34	
	3.	26gm	51	
	4.	27gm	42	
Group – II (standard) diclofenac sodium 5mg/kg	1.	25gm	23	50%
	2.	28gm	19	44.11%
	3.	27gm	26	49.01%
	4.	28gm	17	59.52%
Group - III *Phyla nodiflora* (aerial) low dose (200mg/kg)	1.	26gm	25	45.65%
	2.	25gm	27	20.58%
	3.	27gm	32	37.25%
	4.	26gm	21	50%
Group – IV *Phyla nodiflora (aerial) high dose (400mg/kg)*	1.	29gm	29	36.95%
	2.	30gm	18	47.05%
	3.	26gm	25	50.98%
	4.	26gm	20	52.38%
Group – V *Phyla nodiflora* (root) low dose (200mg/kg)	1.	27gm	26	43.47%
	2.	26gm	27	20.58%
	3.	25gm	36	29.41%
	4.	28gm	33	21.42%
Group – VI *Phyla nodiflora* (root) high dose (400mg/kg)	1.	29gm	30	34.78%
	2.	30gm	26	23.52%
	3.	27gm	32	37.52%
	4.	26gm	27	35.71%

aerial and roots of plant at low dose of 200mg/kg and high dose of 400mg/ kg showed a significant analgesic activity when compared to the standard Diclofenac sodium 5mg/kg. The results of acetic acid induced method of aqueous extract of *phyla nodiflora* linn are shown in Table 3.

CONCLUSION

The dried coarse powder of *phyla nodiflora* linn root and aerial part of the plant was subjected to aqueous solvent extraction process by cold maceration method. Then the extracts were subjected to qualitative analysis. The preliminary phytochemical investigation of *Phyla nodiflora Linn.* has shown the presence of carbohydrates, proteins and amino acids, flavanoids, glycosides, saponins, phytosterols, alkaloids and terpenoids in the plant extract. In Hot plate method, the aqueous extracts of aerial and roots at low dose of 200mg/kg and high dose of 400mg/kg showed significant analgesic activity when compared to the standard Morphine sulphate 5mg/kg. In Acetic acid induced writhing method**,** the aqueous extract of aerial and root at low dose of 200mg/kg and high dose of 400mg/kg showed significant analgesic activity when compared to the standard Diclofenac sodium 5mg/kg. Both the extracts showed significant peripheral and central analgesic activity. But the aerial part of the extract has shown more analgesic activity than root of the plant.

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