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Review Article

Phytochemical and pharmacological studies of Phyla Nodiflora (Verbenaceae): a review

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ABSTRACT

Phyla nodiflora (Verbenaceae) has been used in folk medicine for various ailments such as asthma, bronchitis, knee joint pain, gonorrhea, and irritation of internal hemorrhoids, cardiopathy, hepatitis and fever. It is known to have various biological activities such as antimicrobial, antitumor, anti-inflammatory, antidiabetic, antimelanogenesis, hepatoprotective and antioxidant effects. Phyla nodiflora is a common ingredient of herbal tea used for the treatment of inflammation, menstrual disorders, and infectious disease. In the present study the plant has been completely reviewed for detection and isolation of secondary metabolites and biological activities which will facilitate the scientists to plan for future studies.

Keywords: *Phyla nodiflora,* antibacterial activity, phytochemicals, anti-inflammatory activity

INTRODUCTION:

Phyla nodiflora is a member of family Verbenaceae. The family includes 75 genera and about 2500 species and the genus *Phyla* include 10 species. The specie under study is very commonly found in wet places almost throughout Pakistan plains, often in gregarious patches. *Phyla nodiflora* is mainly distributed in North and Central America, in warmer parts of Asia and Africa, throughout India, Srilanka, Baluchistan and it is native of Calofornia. Aerial parts of this plant are used in the treatment of indigestion in children; its decoction is considered as cooling agent and used as demulcent in cases of venereal diseases (Ali *et al.,* 1974). Synonyms of *Phyla nodiflora* are *Lippia nodiflora, Lippia incisa* and *Phyla incisa. Phyla nodiflora* is known by the local people as Jal papli, *Lippia*, Frog fruit and Bukkhan. *Phyla nodiflora* is fast growing perennial prostate herb. Leaves; obovate, obtuse, somewhat fleshy, and rarely subacute. Their surface is covered with fine hairs and color is grayish green. Leaves are arising in pairs from the stem. Young stem is green to purple in color and becomes grey and woody when mature. Thickness of young stem is 2-3 mm. Flowers are white, rarely pinkish to purple in color, 3 mm long. Mature flowers are tubular at the base, ending in two lipped calyx. The lower lip has two lobes and upper lip has three lobes. Seeds not easily visible to naked eye. Fruits; ovate, 16 mm long and release two brown color mature seeds on maturity (Ali *et al.,* 1974; Ranghunatha, 2003).

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Preliminary phytochemical analysis

Phyla nodiflora contains variety of phytochemical constituents such as alkaloids, glycosides, flavonoids tannins, phenolic compounds, steroids, terpenoids, carbohydrates, proteins, amino acids, gums and mucilage. Flavonoids are present as major component.

Phytochemistry

Halleridone (**1**) and Halleron (**2**) were isolated from dichloromethane and methanol extract of leaves of *Phyla nodiflora* as taxonomic markers (Ravikanth *et al.*, 2000). Eupafolin (**3**), a skin whitening agent isolated from methanol extract of dried aerial part of *Phyla nodiflora*, down regulates melanogenesis (Yen *et al.*, 2012).

Flavanoids, such as hispidulin (**4**), eupafolin (**3**), nodifloretin (**5**) has been reported from methanol extract of whole plant of *Phyla nodiflora*. (Ko *et al.*, 2013; Barua *et al.*, 1971). Ethanol extract of whole plant of *Phyla nodiflora* has been reported to contain lippiflorin, nodifloridin, jaceosidin (**6**), nepetin (**7**) and batatifolin (**8**) (Joshi 1970; Nair *et al.*, 1973) whereas 6-hydroxyluteolin (**9**) and luteolin-7-O-glucoside (**10**) were found to be present in the flowers of *Phyla nodiflora* (Barnabas, *et al.*, 1980). Mono and disulphates of nepetin, hispidulin, jacoesidin, 6-Hydroxyleutolin and nodifloretin were also isolated from the aerial parts of *Phyla nodiflora* (Tomas *et al.*, 1987). Ecteoside (**11**) has been isolated from ethanol extract of whole plant of *Phyla nodiflora* (Khalil *et al.*, 1995). Methyl salicylate (**12**), eugenol (**13**), α-copaene (**14**), β-bisabolene (**15**), γ-sitosterol (**16**) and stigmasterol (**17**) have been identified from methanol extract of whole plant of *Phyla nodiflora*. (Ko *et al.*, 2013). Steam distillation of *Phyla nodiflora* extracts showed the presence of volatile constituents including mixtures of hydrocarbons and oxygenates. The major components are β carbolene (**18**), methyl salicylate (**12**), linalool (**19**) and Cymen-8-ol (**20**) (Elakovich and Stevens 1985).

Ethnomedicinal/ traditional uses

Phyla nodiflora plant is appetizing, stomachic, constipating, anthemintic, vulnerary, aphrodisiac, opthelmic, diuretic, alexeteric and febrifuge. It is useful in vitiated conditions of *Pitta*, burning sensation, anorexia, flatulence, colic, dyspepsia, helminthiasis, diarrhea, ulcer, strangury, asthma, bronchitis, knee joint pain, gonorrhea, irritation of internal hemorrhoids, cardiopathy, hepatitis and fever (Raghunatha, 2003). In Taiwan *Phyla nodiflora* is used as an herbal drink, a nourishing agent, immunomodulator and anti-inflammatory agent to prevent many diseases (Yang, *et al.*, 1998). Ethno pharmacological applications of *Phyla nodiflora* have been mentioned for curing many skin diseases. *Phyla nodiflora* is used for curing pimples, carbuncle and skin diseases in folk cosmetics (Abbasi *et al.*, 2010). Traditionally *Phyla nodiflor*a is used as diuretic astringent to bowels, maturant, stomachic, and useful in fever and cold, lack of bowel movements, bronchitis and hypertension by the local and tribal peoples of South India. Antimalarial activity was reported and the herb also poses cooling and diuretic activities and useful in the treatment of knee joint pain. Leaves of *Phyla nodiflora* have antioxidant, antipyretic, anti-inflammatory and analgesic activities. Pain in knee joints and lithiasis (Durairaj *et al.*, 2008). *Phyla nodiflora* is the simple siddha remedy for hizhuvettu (alopecia area) (Panniachary *et al.*, 1989).

PHARMACOLOGICAL ACTIVITIES

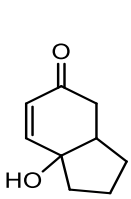
Antimelanogenisis activity

Eupafolin isolated from methanol extract of dried aerial parts of *Phyla nodiflora* is responsible for inhibiting tyrosine activity to down regulate melanogenesis in a dose dependent manner (0.1-10μM) (Ko *et al.*, 2013 and Yen *et al.*, 2012). Hyperpigmentation is caused by overproduction of tyrosinases enzymes including TYR, TRP-1 and TRP-2 which leads to melasma (Hearing and Tsukamoto 1991).

Cytotoxic activity

Halleridone and Halleron isolated from dichloromethane and methanol extracts of the leaves of *Phyla nodiflora* are known to have anticancer, antitumor and cytotoxic activities (Ravikanth *et al.*, 2000). The methanol extract of whole plant of *Phyla nodiflora* was evaluated for antitumor effect using Erich's ascites carcinoma (EAC) in Swiss albino mice at 200 and 400 mg/ kg of body weight. The extract was found to decrease tumor cell volume, cell count and packed cell volume. The hematological parameters did not significantly altered at these specified doses but the levels of catalase, reduced glutathione and superoxide dismutase were increased and lipid peroxidation reduced which indicated that the

anti-tumor activity of methanol extract of whole plant of *Phyla nodiflora* was due to the presence of antioxidant components (Durairaj *et al.*, 2009).



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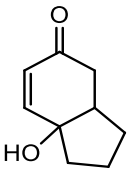

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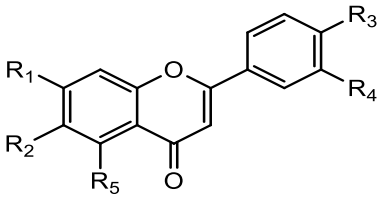

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3	OH	OCH_3	OH	H	H
4	OH	OCH_3	OH	OH	H
5	OH	OH	OH	OCH_3	H
6	OH	OCH_3	OH	OCH_3	OH
7	OH	OCH_3	OH	OH	OH
8	OH	OH	OH	OCH_3	OH
9	OH	OH	OH	OH	OH
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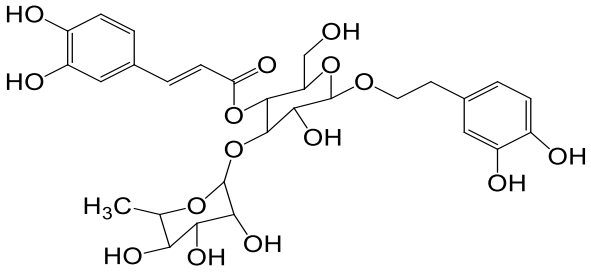

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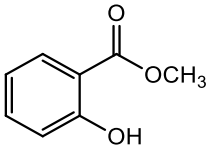

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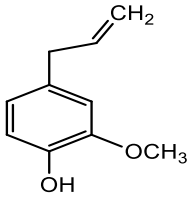

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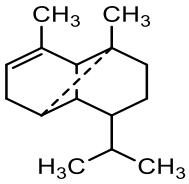

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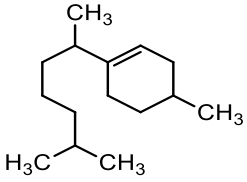

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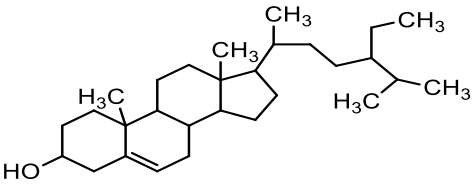

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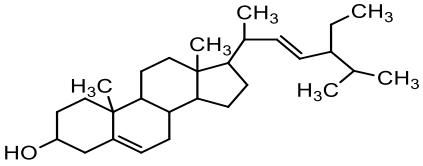

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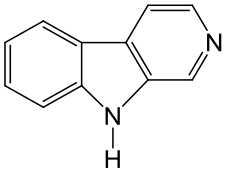

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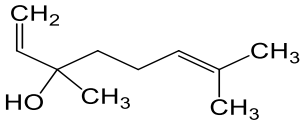

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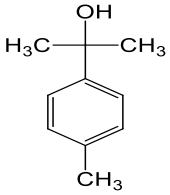

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Structures of compounds isolated from *Phyla nodiflora*

Diuretic activity

Diuretic potential of methanol and aqueous extracts of dried aerial parts of *Phyla nodiflora* was determined using *in-vivo* Lipschitz test model in albino rats. Furosemide was used as a standard to measure the parameters like increase in urine volume and electrolyte excretion. Both extracts have significant diuretic activity at 500mg/kg body weight (Shukla *et al.*, 2009).

Antibacterial activity

The ethanol extract of whole plant of *Phyla nodiflora* and its sub fractions (n-hexane, chloroform, ethyl acetate, n-butanol and aqueous) were subjected to antibacterial assay against seven bacterial strains i.e. *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus aureus and Bacillus subtilis.* Ethyl acetate and chloroform fractions at concentration of 20 mg/ml showed excellent activity against *Bacillus subtilis*, *Staphylococcus epidermidis and Staphylococcus aureus.* Ethyl acetate fraction showed maximum zone of inhibition (16mm) for *Salmonella typhi* and chloroform fraction showed maximum zone of inhibition (18mm) for *Staphylococcus aureus.* The details of antibacterial inhibition are shown in Table-1. (Zakir *et al.,* 2013). The methanol extracts of seeds of *Phyla nodiflora* exhibits antibacterial activity against *Bacillus subtilis*, *Styphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* (Patel *et al.,* 2011). Hexane, chloroform and alcohol extracts of leaf of *Phyla nodiflora* were evaluated for antibacterial activity against *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Shigella flexneri and Salmonella Paratyphi A.* The chloroform extract showed maximum zone of inhibition (13mm) for *Bacillus subtilis* and alcohol extract showed maximum zone of inhibition against *Bacillus cereus* (11mm). Some anti bacterial activity have been reported in Table 1 and Table 2.

Table 1: Antibacterial activities of ethyl-acetate and chloroform fractions of etnanol extract of *Phyla nodiflora*

Microorganism (bacterial strains)	Zone of inhibition (mm)	
	Ethyl acetate fraction	Chloroform fraction
Escherichia coli	0.0	0.0
Pseudomonas aeruginosa	0.0	0.0
Salmonella typhi	16	13
Staphylococcus aureus	11	18
Staphylococcus epidermidis	7	8
Klebsiella pneumoniae	5	0.0
Bacillus subtilis	11	11

Table 2: Antibacterial activity of hexane, chloroform and alcohol extracts of leaf of *Phyla nodiflora*

Name of bacteria	Zone of inhibition (mm)		
	Hexane extract	Chloroform extract	Alcohol extract
Bacillus subtilis	9	13	9
Bacillus cereus	9	9	9
Salmonella Paratyphi A	9	11	8
Staphylococcus aureus	7	10	9
Shigella flexneri	9	11	9

Antifungal activity

The ethyl acetate, ethanol, methanol and water extracts of whole plant of *Phyla nodiflora* had been screened for antifungal activity against *Aspergillus niger, Aspergillus flavus, Paecilomyces varioti, Microsporum gypseum, Trichophyton rubrum.* Ethanol extract showed 100 % inhibition against tested organism as compared to aqueous (82.6%), methanol (61 %), ethyl acetate (87 %) extracts (Pascual *et al.,* 2001; Pirzada *et al.,* 2005).

Antiproliferative and apoptotic effect

The methanol and ethyl acetate extracts of leaves and stems of *Phyla nodiflora* have antiproliferative and apoptotic effects on human breast cancer cell line. The extracts at 90-120µg/ml were capable of inhibiting cancer cell growth via apoptosis (Rabi'atul'adawiyah *et al.,*2014).

Antidiabetic and hypolipidemic effect

γ – sitosterol isolated from methanol extract of *Lippia nodiflora* was screened for antidiabetic potential. γ- sitosterol at 20 mg/kg body weight was administered to streptozotocin induced diabetic rats. A decrease in glycosylated hemoglobin and blood glucose level with significant increase in plasma insulin, body weight and food intake were observed. Furthermore γ-sitosterol had also been evaluated for antihyperlipidemic effect. The compound was

responsible for decreasing serum cholesterol, very low density lipoprotein and triglycerides levels with elevated level of high density lipoprotein which indicated its hepatoprotective effect (Rangachari and Savarimuthu 2011).

Anti-inflammatory activity

Methanol extract of whole plant of *Lippia nodiflora* also exhibited anti-inflammatory and noceptive activities at 20 µg/ml. Cyclo-pentano phenatherol isolated from methanol extract of *Lippia nodiflora* showed inhibition of COX-2 and prostaglandin biosynthesis at 10 µg/ml and thus, it was concluded that *Phyla nodiflora* have potential to cure inflammation (Durairaj *et al.,* 2007; Ahmad *et al.,* 2004).

Neuropharmacological effects

Petroleum ether, chloroform and methanol extracts of aerial parts of *Phyla nodiflora* were evaluated for neuropharmacological effects. Diazepam was used as standard drug to monitor the parameters like sleeping time, locomotor activity, exploratory behavior pattern, motor coordination and convulsions. Flavonoids present in ethanol and chloroform extract were found to be responsible for central inhibitory, anticonvulsants and anxiolytic effects at 500mg/kg. Petroleum ether extract showed absence of flavonoids thus did not produce any central inhibitory effect (Kumaresan *et al.,* 2011).

Antiurolithiatic activity

The ethanol extract of *Phyla nodiflora* was studied for investigation of antiurolithiatic activity against most common calcium oxalate type of kidney stone. Gentamycin and calculi producing diet was administered for producing calcium oxalate urolithiasis. The extract was also accessed for *in vivo* antioxidant parameters like catalase, lipid peroxidation and reduced glutathione and *in vitro* scavenging of nitric oxide and free radicals that helps in preventing calcium oxalate type stone formation and dissolving of preformed stones in kidney. The extract was found to be safe up to maximum dose of 8g/kg (Dodala *et al.,* 2010).

Hepatoprotective and antioxidant potential

The methanol extract of whole plant of *Lippia nodiflora* has been evaluated for antioxidant activity and hepatoprotective effects in paracetamol induced liver injury (750mg/kg, body weight). The extract was orally administered for 7 days. A significant decrease in the level of blood serum enzymes such as serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), aspartate transaminase (ALP), bilirubin and lipid peroxidation was observed. The hepatoprotective effect of *Lippia nodiflora* was found to be equivalent to that of standard silymarin 25mg/kg (Mazumde *et al*., 2008).

CONCLUSION

Phyla nodiflora is distributed worldwide. It is used as traditional medicine in many regions. Many numbers of compounds have been isolated and the major components present are flavonoids. Flavonoids have many important pharmacological effects so it can further investigated for more biological activities which contributes towards its future prospects for its use in pharmaceutical industry and curing of various ailments.

REFERENCES

Ali SI, Nasir E and Stewart H (1974). Flora of West Pakistan. *Department of Botany, University of Karachi,* 77: 1-40.

Raghunatha SI (2003). Indian medicinal plants, a compandium of 500 species 4: 1-444.

Ravikanth V, Ramesh P, Diwan PV and Venkateswarlu Y (2000). Halleridone and Hallerone from *Phyla nodiflora* as taxonomic markers. *Biochem. Syst. Eco.*, 28: 905-906.

Yen FL, Wang MC, Liang CJ, Ko HH and Lee CW (2012). Melanogenesis Inhibitor(s) from *Phyla nodiflora* extract. *Evidence-Based Complement. Altern. Med.*, 1-9.

Ko HH, Chiang YC, Tsai MH, Liang CJ, Hsu LF, Shu YL Wang MC, Yen FL and Lee CW (2013). Eupafolin, a skin whitening flavonoid isolated from *Phyla nodiflora*,

downregulated melanogenesis: Role of MAPK and Akt pathways. *J. Ethnopharmacol.,* 151: 386-393.

Barua AK, Chakrabarti P and Sanyal PK (1971). Structure of nodifloretin new flavone from *Lippia nodiflora. Trasanc. bose res. inst.* 3: 5-8

Joshi BC (1970). Chemical examination of *Lippia nodiflora.* Vijnanaparishad anusandhan patrika, 4: 214-219.

Nair AG, Ramesh P, Nagarjan S and Subraimanam SA (1973). New flavone glycosides from *Lippia nodiflora. Indian J. chem.,* 2: 1316- 1317.

Barnabas C, Gunasingh G and Nagarajan S (1980). Flavonoids from the flowers of *Phyla nodiflora* Linn. *Indian J. chem.,* 9: 822

Tomas BF, Harborne JB and Self R (1987). Twelve 6-oxygenated flavones sulphates from *Lippia nodiflora. J. pharm. Sci.,* 2: 256-265.

Khalil AT, Lahloub MF and Salma OM (1995). Phenolic compounds from *Lippia nodiflora. J. pharm. Sci.,* 2: 256-265.

Elakovich SD and Stevens KL (1985). Volatile constituents of *Lippia nodiflora. J. Nat. Prod.* 3 : 504-506.

Yang YP, Lu SY and Chen TT (1998). Verbenaceae. 127: 421

Abbasi AM, Khan M, Ahmad M, Zafar S, Jahan and Sultana S (2010). Ethnopharmacological applications of medicinal plants to cure skin diseases and in folk cosmetics among the tribal communities of North-West Frontier Province, Pakistan. *J. Ethnopharmacol.,* 128: 322-335.

Durairaj AK, Vaiyapuri TS, Kanti MK and Malaya G (2008). Protective activity and antioxidant potential of *Lippia nodiflora* extracts in paracetamol induced hepatotoxicity in rats. *Iranian J. Pharmacol. Res.,* 7: 83-89.

Panniachary G, Rajalakshmi S, Saroja PR, Sundaram M and Veluchamy G (1989). Simple siddha remedy for hizhuvettu(alopecia area) a pilot study. *J. Res. Ayurveda and Siddha,* 10: 1-2.

Hearing VJ and Tsukamoto K (1991). Enzymatic control of pigmentation in mammals. FASEB J, 5: 2902-2909.

Durairaj AK, Mazumdar UK, Gupta M and Selvan VT (2009). Effect on inhibition of proliferation and antioxidant enzyme level of *Lippia nodiflora* in EAC cell line treated mice. *J. Comp. Integ. Med.,* 1: 713-714

Shukla L, Patel R and Kukkar R (2009). Study of phytochemical and diuretic potential of methanol and aqueous extracts of aerial parts of *Phyla nodiflora* Linn. *Int. J. Pharmacy Pharm. Sci.,* 1: 85-91.

Zakir U, Rehman A, Najeeb U, Shakeel AK, Shahid UK and Ahmad I (2013). Antibacterial study of *Phyla nodiflora* Linn. *J. Chem. Pharm. Res.,* 5: 86-90.

Patel JB, Shah KH and Patel RC (2011). Evaluation of anti-bacterial activity of methanolic extract of seeds of *Phyla nodiflora* Linn. *Int. Res. J. Pharm.,* 6: 91-93.

Pascual ME, Slowing K, Carreto E, Sanchez MD and Villar A (2001). *Lippia*: traditional uses,chemistry and pharmacology - a review. *J. Ethnopharmacol.,* 3: 201-214.

Pirzada AJ, Iqbal P, Shaikh W, Kazi TJ and Ghani KV (2005). Studies on elemental composition and antifungal activity of medicinal plant *Lippia nodiflora* against skin fungi. *J. Pak. Assoc. Derma*., 2: 113-118.

Rabi'atul'adawiya MA, Cheong BE and Teoh PL (2014). Antiproliferative and apoptotic effects of *Phyla nodiflora* extracts on human breast cancer cell line. *Asian Pacific J. Trop. Disease.,* 4: 238.

Rangachari B and Savarimuthu I (2011). Antidiabetic and Hypolipidemic effect of methanol extract of *Lippia nodiflora* in streptozotocin induced diabetic rats. *Asian pacific J. Trop. Biomed.,* 1 : 30-36.

Ahmad F, Salim MS, Das AK and Chaudhari MSK (2004). Anti-inflammatory and noceptive activities of methanolic axtract of *Lippia nodiflora*. *Die pharmazie.,* 4: 329-330.

Durairaj AK, Mazumdar UK, Gupta M and Selvan VT (2007). Antimicribial and lipid peroxide scavenging activity of *Lippia nodiflora* (Verbenaceae). *Pharmacology online.,* 3: 177-189.

1. Kumaresan P, Thirupathy ST and Vijay C (2011). Neuropharmacological activity of *Lippia nodiflora* Linn. *Pharmacog. Res.,* 3: 194-200.
2. Dodala S, Diviti R, Koganti B and Prasad (2010). The effect of ethanolic extract of *Phyla nodiflora L* Greene against calculi producing diet induced urolithiasis. *Indian J. Nat. Prod. Resour.,* 3: 314-321.
3. Mazumdar UK, Durairaj AK, Gupta M and Selvan VT (2008). Protective activity and antioxidant potential of *Lippia nodiflora* extract in paracetamol induced hepatotoxicity in rats. *Iranian J. Pharmacol. Ther.,* 1: 83-89.