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Phytochemical screening and Anti-oxidant activity of *Ammannia octandra* L.f

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ABSTRACT

The investigation includes phytochemical screening of different extracts of *Ammannia octandra* L.f. Petroleum ether, Chloroform, Ethyl acetate, Ethanol as well as the aqueous extracts of *Ammannia octandra* L.f showed many phyto-constituents. The phytoconstituents of *Ammannia octandra* L.f were found to be glycosides, alkaloids, flavanoids, tannins and phenolic compounds. Antioxidant activity was determined for three extracts (Petroleum ether, ethyl acetate and ethanolic). DPPH assay, Superoxide free radical scavenging activity, Nitric oxide radical scavenging activity, Hydroxyl radical scavenging activity as well as Total antioxidant activity methods were followed for antioxidant assays. The ethanolic extract showed better antioxidant activity. Pharmacological screening of different therapeutic activities of *Ammannia octandra* L.f has to be carried out so that it may be used in future as a good natural antioxidant source.

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INTRODUCTION

Species of independent existence which contains unpaired electrons are said to be free radicals. These unstable species reacts instantaneously with other molecules by giving or taking electrons. They can react with carbohydrates, proteins, lipids and DNA (Salunkhe *et al.*, 1996; Pal *et al.*, 2009). They may be nitrogen derived or oxygen-derived. Examples of oxygen-derived ones aresuperoxide [O_2^-], hydroxyl [OH]l, hydroperoxyl[HO_2,]peroxyl[ROO]and alkoxyl[RO] radicals. Hydrogen peroxide oxygen is a non-radical. Nitrogen derived species are nitric oxide [NO], peroxynitrate [ONOO], nitrogen dioxide [NO_2] and dinitrogen trioxide [N_2O_3] (Badarinath *et al.*, 2010; Lobo *et al.*, 2010). Trapping free radicals is the main feature of antioxidants. The normal process of metabolism produces free radicals in living systems. Free radicals can cause many pathological conditions like anemia, arthritis, neurogeneration, asthma, mongolism, Parkinson's diseases, inflammation, dementia and ageing process (Ara and Nur, 2009; Thangavelu and Thomas, 2010). These pathological conditions due to free radicals are prevented by antioxidants with their radical scavenging activity. Antioxidants obtaining from natural sources are always in demand (Gini *et al.*, 2017). Antioxidants play a major role in the defense mechanism to safeguard our body from various diseases. Studies confirmed that a major source of antioxidants are from plant orgin. Some of them are flavanoids, flavones, isoflavones, lignins, anthocyanins, catechins, coumarins and isocatechins. Many synthetic antioxidants reported serious side effects like carcinogenesis and damage of liver. Example; (BHA) butylated hydroxyanisole,

(PG) propyl gallate, butylated hydroxytoluene and test butylated hydroquinone Therefore there is a need to replace synthetic antioxidants with natural antioxidants having less side effects.

Biological and environmental diversity of India is great when compared with the rest of the world. *Ammannia octandra* L.f. Belong to the family Lythraceae, robust herbs up to 50cm high. Leaves oblong-lanceolate, 4.5cm long and 0.7cm wide, glabrous, chartaceous, base auriculate-subcordate, margin entire, apex acuminate. Cymes simple, axillary; pedicel to 1mm. Flowers- 7mm across, calyx-tube 4mm, angular margins sharp, lobes 4, 1mm. Four pellets, flame-coloured, with a darker midnerve, orbicular,4mm, fugacious, crumpled, stamens 4+4, inserted at different levels on calyx-tube; filaments to 6mm, ovary 1.5mm, 4-celled; style 5mm, fruit capsule 4.5mm, equalling the thick calyx-tube, seeds turgid. *Ammannia octandra* L.f is distributed in south and South East Asia. In India, it is found in Andra Pradesh, Kerala and Tamilnadu. This species is found in plains, especially towards the coast in low-lying moist areas, fallow fields and as a weed in paddy fields (Ho and Chan, 2002). The present study focused to explore the valuable phytoconstituents of *Ammannia octandra* L.f and its antioxidant properties.

MATERIALS AND METHODS

Plant material and preparation of extracts

*Ammannia octandra*L.f. Plants were collected from Thirunelveli District of Tamilnadu, India. Identification and authentification of the plant done by Mr Chelladurai, Research officer-Botany, Central council for research in Ayurveda and Sidha, Government of India. The parts of the plant were gabled for the elimination of contaminants dried in the shade and powdered. Plant material of about 300gm (powdered) was used for extraction using soxhelet extractor. For successive extraction, the solvents used are petroleum ether, Chloroform, Ethyl acetate and Ethanol. For aqueous extraction, method of hot percolation was used (48hours). Different extracts were concentrated with the aid of rotary evaporator, weighed, properly labeled and stored for further use in refrigerator (Evans, 2002; Kokate and Purohit, 2010).

Preliminary Phytochemical Screening of Plant Extracts

Qualitative tests on every extract of *Ammannia octandra*L.f. Performed for phytoconstituents present. The results of chemical tests are presented in Table 1 (Harborne, 1998; Mukherjee, 2002; Pvman, 1931; Middeltone, 1952; Kokate *et al.*, 2002; Peach and Trancey, 1955; Shellard, 1957).

Antioxidant assays

DPPH photometric assay

Measurement of absorption of DPPH solution carried out at 517nm after addition of antioxidant. The absorption was decreased, and the reference used was ascorbic acid (10mg/ml DMSO). Stable free radical-1, 1-diphenyl-2-picryl hydrazyl is red in colour and turns yellow on scavenging. This character of DPPH assay is used to show free radical scavenging activity. The scavenging between (H-A) antioxidant and(DPPH) is written as, [H-A]+ DPPH$\rightarrow$ (A) + DPPH-H. The absorbance decreases as a consequence of the reaction of antioxidants with DPPH. Scavenging potential of extracts or antioxidants in terms of their hydrogen donating ability is indicated by the degree of discoloration. Extracts of volumes (1.25 to10μl) were made to 40μl using DMSO, and then 2.96ml DPPH (0.1mM) was added. Incubation of reaction mixtures carried out at room temperature in dark condition. The absorbance of the mixture after 20 minutes was observed at 517nm. DPPH (3ml) taken as control (Halliwell and Gutteridge, 1990; Mensor *et al.*, 2001).

Superoxide free radical scavenging activity

Hydroxyl radical, as well as singlet oxygen, can be formed from superoxide and hence is biologically important. In riboflavin-NADH system, anion superoxide is generated by NADH oxidation. Blue formazan product is formed in this assay due to the reduction of NBT.Extracts of different volumes (62.5 to 2000μg/ml), 0.05ml solution of Riboflavin (0.12mM), 0.2ml solution of EDTA [0.1M], 0.1ml solution of Nitro-blue tetrazolium(NBT) were taken in test tubes and mixture was diluted using phosphate buffer to 2.64ml [0.067M]. Using DMSO as the blank, optical density of solution measured at 560nm after 5minutes illumination. The difference in optical density determined after 30minutes incubation, in fluorescent light. After 30minutes illumination, optical density was measured at 560 nm in UV visible spectrophotometer (Valentão *et al.*, 2001; Winterbourn *et al.*, 1975).

Nitric oxide radical scavenging activity

In aerobic conditions, nitric oxide is very unstable species. Nitric oxide reacts with oxygen to produce stable nitrates and nitrites. Here intermediate NO_2 and $N_3O_4$are formed. Garrat method is used for determination (Garratt and Johnson, 1964). In the presence of scavenger, i.e., the test compound, the quantity of nitrous acid decreases. UV visible spectrophotometric method was used for measuring

nitric oxide scavenging activity Different volumes of the extract (62.5-2000μg/ml)prepared in methanol was mixed with Sodiumnitroprusside (5mmolL-1) in phosphate-buffered saline of pH7.4and incubated at 25°C for 30min. A control was prepared without the test compound, but an equal volume of methanol was used. 1.5ml of the incubated solution was removed after 30 minutes and diluted with 1.5ml of Griess reagent (2% phosphoric acid+1% sulphanilamide+0.1% N-1naphthyl ethylene diamine dihydrochloride). Chromophore was formed in diazotization (nitrate with sulphanilamide) and coupling with N-1 naphthyl ethylenediamine dihydrochloride. Chromophore absorbance measured at 546nm and percentage scavenging activity determined (Shirwaikar *et al.*, 2006).

Hydroxyl radical scavenging activity method

The method was based on quantitative measurement. A degradation product is formed when 2 deoxyribose condenses with TBA. Radical named hydroxyl was generated by the Fenton reaction (Fe^{3+} -ascorbate- EDTA -H_2O_2 system). Final volume of 1 ml reaction mixture contained the following; 2 deoxy 2 ribose (2.8mM), $FeCl_3$(100μM), EDTA(100μM), H_2O_2(1.0mM), ascorbic acid(100μM) in buffer KH_2PO_4-KOH (20mM pH 7.4) and various volumes (62.5 – 2000μg/ml) of test sample. After incubation (37°C)for 1hour, the reaction mixture of 0.5ml was added to1ml of 2.8percentage TCA, then 1ml aqueous TBA was added, and the mixture was incubated (15 minutes)at 90°C to develop the colour. The absorbance measured at 532nm after cooling, against the blank solution.

Total antioxidant activity method

Extracts of 0.3ml (sample) was combined with 3.0ml of reagent (4mM ammonium molybdate +0.6ml H_2SO_4+28mM sodium phosphate). The reaction solutions containing tubes were incubated at 95^{0}C. After 90 minutes, cooled to room temperature and optical density measured against blank at 695nm. Blank used was methanol (0.3ml) instead of extract. The activity in terms of a number of gram equivalent of standard ascorbic acid is expressed.

Statistical Analysis

Mean (±) SD calculated and were expressed in analytical results. Measurements were replicated three times. Calculations are based on the regression analysis method.

RESULTS AND DISCUSSION

Phytochemical screening of *Ammannia octandra* L.f

Table 1 shows the phytoconstituents of various extracts(petroleum ether, chloroform, ethyl acetate, ethanol and aqueous) of *Ammannia octandra*L.f. Preliminary phytochemical analysis showed presence of glycosides, alkaloids, amino acids and tannins in petroleum ether extract, glycosides, alkaloids and amino acids in chloroform extract, alkaloids, flavanoids, tannins and phenolic compounds in ethyl acetate extract, glycosides, alkaloids, flavanoids, tannins and phenolic compounds in ethanolic extract, alkaloids, flavanoids and tannins in aqueous extract.

Antioxidant activities of selected extracts of *Ammannia octandra* L.f.

The in-vitro antioxidant activity of petroleum ether, ethyl acetate and ethanol extracts by five different methods were conducted as per the procedures mentioned. Different concentrations (62.5-2000μg/ ml) of plant extracts were used to determine the antioxidant activity and 50% growth inhibition (IC_{50}). The results showed that with increasing concentration of extracts, the percentage of growth inhibition is also increasing. Results are given in Tables 2, 3, 4, 5 and 6 and graphically in Figures 1, 2, 3, 4 and 5.

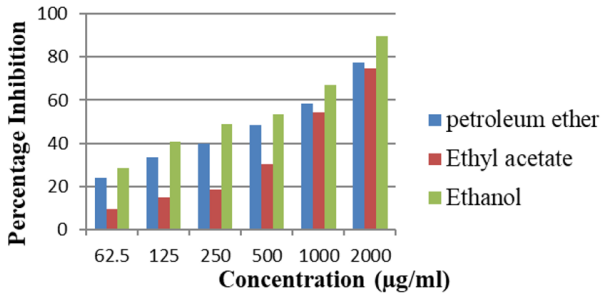

Figure 1: Comparative study of % Inhibition of different extracts of *Ammannia octandra* L.f-DPPH assay method

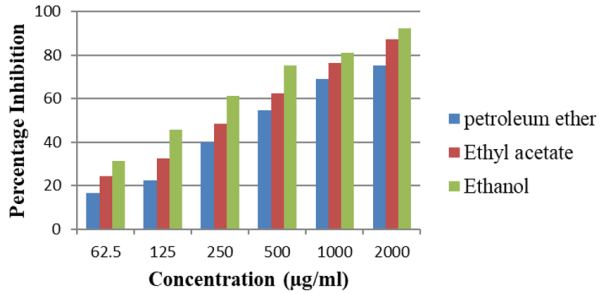

Figure 2: Comparative study of % Inhibition of different extracts of *Ammannia octandra* L.f-superoxide free radical scavenging method

Phytochemical screening clearly indicated the presence of glycosides, alkaloids, amino acids, fla-

Table 1: Phytochemical screening of *Ammannia octandra* L.f

Sl.No	Tests	Petroleum ether	Chloroform	Ethyl Acetate	Ethanol	Water
1	Carbohydrates	-	-	-	-	-
2	Glycosides	+	+	-	+	-
3	Saponins	-	-	-	-	-
4	Alkaloids					
	Dragendorffs test	+	+	+	+	+
	Tannic acid test	+	+	+	+	+
	Wagners test	+	+	+	+	+
5	Flavonoids	-	-	+	+	+
6	Phenolic compounds	-	-	+	+	-
7	Tannins	+	-	+	+	+
8	Steroids and terpenoids	-	-	-	-	-
9	Proteins	-	-	-	-	-
10	Aminoacids	+	+	-	-	-
11	Fixed oils and fats	-	-	-	-	-

Table 2: Percentage inhibition of extracts of *Ammannia octandra* L.f- DPPH method

Concentration (μg/ml)	Percentage Inhibition		
	Petroleum ether	Ethyl acetate	Ethanol
62.5	24.08	9.42	28.6
125	33.62	14.72	40.52
250	39.77	18.42	48.75
500	48.6	30.17	53.6
1000	58.32	54.38	66.85
2000	77.42	74.81	89.85

Table 3: Percentage inhibition of extracts of *Ammannia octandra* L.f- Superoxide free radical scavenging method

Concentration (μg/ml)	Percentage Inhibition		
	Petroleum ether	Ethyl acetate	Ethanol
62.5	16.44	24.23	31.2
125	22.4	32.5	45.5
250	39.64	48.57	61.27
500	54.72	62.48	75.18
1000	69.12	76.5	81.15
2000	75.12	87.24	92.26

vanoids, tannins and phenolic compounds as phytoconstituents in *Ammannia octandra* L.f

Based on the evidenced phytochemical constituents of *Ammannia octandra* L.f, it is vital to evaluate the antioxidant potential. Plants due to their therapeutic principles are considered as an important source of remedy since ancient era. Hence it is important to determine the free radical scavenging activity. One of the stable free radical that has been commonly utilized to prove the antioxidant activity of various natural products is DPPH. The more antioxidants present in the extract, the more DPPH reduction will occur. Present investigation infers that *Ammannia octandra* L.f has considerable DPPH radical scavenging activity and its maximum was elicited in ethanolic extract. The antioxidant effect of extracts may be due to the neutralization of DPPH free radicals, either by transfer of electrons or by transfer of hydrogen atom.

Table 4: Percentage inhibition of extracts of *Ammannia octandra* L.f- nitric oxide radical scavenging method

Concentration (μg/ml)	Percentage Inhibition		
	Petroleum ether	Ethyl acetate	Ethanol
62.5	24.44	30.44	38.46
125	28.25	34.76	42.68
250	32.15	56.74	59.72
500	57.45	62.48	65.38
1000	67.48	68.18	72.18
2000	75.12	80.75	88.18

Table 5: Percentage inhibition of extracts of *Ammannia octandra* L.f -Hydroxyl radical scavenging method

Concentration (μg/ml)	Percentage Inhibition		
	Petroleum ether	Ethyl acetate	Ethanol
62.5	12.38	18.23	23.65
125	23.48	28.19	36.19
250	54.52	61.38	68.52
500	67.44	75.21	83.15
1000	75.32	83.92	89.58
2000	83.18	89.24	94.53

Table 6: Percentage inhibition of extracts of *Ammannia octandra* L.f-Total antioxidant activity method

Concentration (μg/ml)	Percentage Inhibition		
	Petroleum ether	Ethyl acetate	Ethanol
62.5	7.92	22.4	35.09
125	32.15	36.14	45.34
250	42.13	51.38	55.91
500	50.82	60.43	64.23
1000	63.76	68.28	73.82
2000	72.13	81.35	89.44

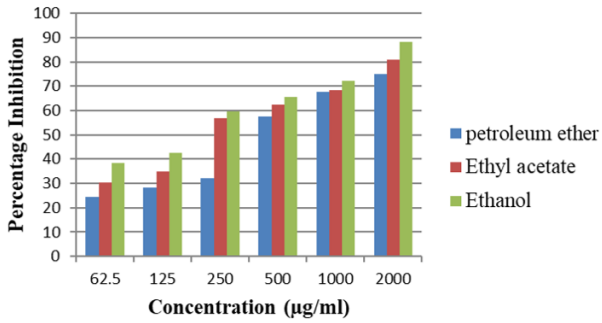

Figure 3: Comparative study of % Inhibition of different extracts of *Ammannia octandra* L.f-nitric oxide radical scavenging method

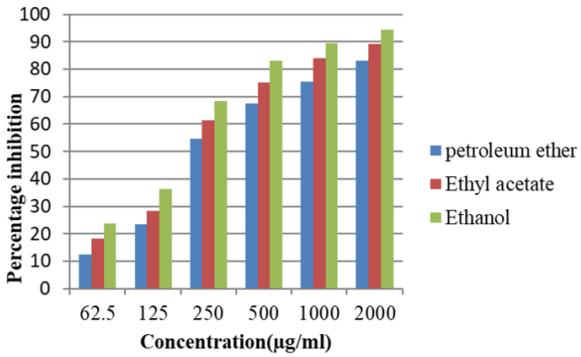

Figure 4: Comparative study of % Inhibition of different extracts of *Ammannia octandra* L.f-hydroxyl radical scavenging method

Superoxide radical is another harmful ROS to cellular components. Superoxide radicals in the biologi-

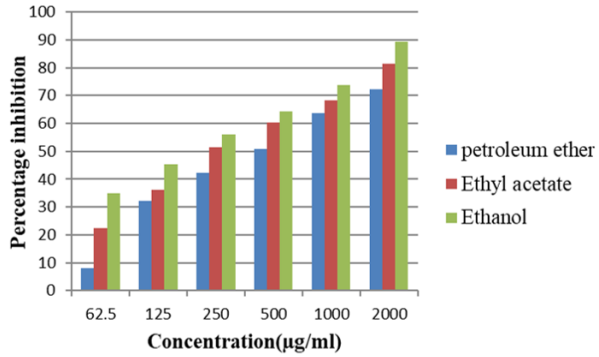

Figure 5: Comparative study of % Inhibition of different extracts of *Ammannia octandra* L.f-Total antioxidant activity method

cal system contribute to tissue injury. This species indirectly initiates the lipid peroxidation by the generation of singlet oxygen (Ramesh and Rao, 2018). Phytomedicine research elicited that the antioxidant property of flavanoids are mainly by scavenging of superoxide anion. Among the three extracts of *Ammannia octandra* L.f, ethanolic and ethyl acetate extracts with minimum IC_{50} values exhibits strong antioxidant activity.

The involvement of nitric oxide in many biological functions is important. It involves neurotransmission, inhibition of platelet aggregation, vascular homeostasis, smooth muscle relaxation, regulation of cell-mediated toxicity and antitumor activities (Hagerman *et al.*, 1998). Other than the beneficial effects of nitric oxide, its contribution to that of oxidative damage also reported. NO itself react with superoxide and form a potent antioxidant, the peroxynitrite anion which will decompose to produce OH and NO. The scavenging of NO by the extracts of *Ammannia octandra* L.f was increased in a concentration-dependent manner from 62.5μg/ml to 2000μg/ml.

Hydrogen peroxide as such is not much reactive. But in a few cases, it causes cell toxicity due to the generation of hydroxyl radical. Hence it is important to eliminate the hydrogen peroxide by antioxidant defense system of a cell (Battu *et al.*, 2011). The IC_{50} value of petroleum ether, ethyl acetate as well as ethanolic extracts by DPPH assay method was found to be 805.00μg/ml, 1138.529μg/ml and 489.285μg/ml respectively. The IC_{50} value of petroleum ether, ethyl acetate as well as ethanolic extracts by superoxide free radical scavenging activity method was found to be 802.857μg/ml, 480.667μg/ml and 131.724μg/ml respectively. The IC_{50} value of petroleum ether, ethyl acetate as well as ethanolic extracts by nitric oxide free radical scavenging method was found to be 763.846μg/ml, 426.818μg/ml and 177.826μg/ml respectively. The IC_{50} value of petroleum ether, ethyl acetate as well as ethanolic extracts by hydroxyl radical scavenging method was found to be 568.387μg/ ml, 362.903μg/ ml and 135.33μg/ml respectively. The IC_{50} value of petroleum ether, ethyl acetate as well as ethanolic extracts by the total antioxidant method was found to be 860.769μg/ ml, 533.60μg/ml and 39.047μg/ml respectively. It is evident from the results that *Ammannia octandra* L.f possesses good scavenging activity with minimum IC_{50} value for the ethanolic extract. The three extracts have shown good hydroxyl radical scavenging activity rendering their utilization in the treatment of various ailments associated with oxidative stress (Liu *et al.*, 2008). Antioxidant activity was carried out at six different concentrations of plant extracts to determine the IC_{50}. Highest antioxidant activity was with ethanolic extract of *Ammannia octandra* L.f. The growth inhibition percentage of plant extracts showed a significant increase in higher concentrations.

CONCLUSION

The plant *Ammannia octandra* L.f showed many phytoconstituents. They are glycosides, alkaloids, phenolic compounds, flavanoids and tannins. The study concludes that the petroleum ether, ethyl acetate as well as ethanolic extracts of the plant *Ammannia octandra* L.f posses antioxidant activity. The ethanolic extract has good antioxidant activity when compared to others. Column chromatography studies are to be conducted for isolation and further characterization of biologically active compounds. The traditional uses of other species of *Ammannia* show good pharmacological effects. *Ammannia octandra* L.f also contains phytochemicals which are antioxidant in nature.

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