INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

COMPARITIVE IN - VITRO ANTIOXIDANT STUDY OF THREE SPECIES FROM EUPHORBIACEAE FAMILY

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ABSTRACT

Phyllanthus niruri, Emblica officinalis and *Saropus androgynous* are the plants which are traditionally used for many siddha and ayurvedic formulations.Phylanthus niruri is having good hepato protective activity. These three plants are having antimicrobial activity and also the chemical constiruents like vitamim-c ,tannins and flavonoidds are present. In the present study, the physico chemical parameters like ash values, Extractive values and total fiber contents were estimated. A comparative approach of the antioxidant activity of the three plants, the vitamin C estimation by chemical assay method. The flavonoids were isolated and their RF values were calculated by HPTLC method, and it was compared with that of the standard ascorbic acid and epicatechine. In this study the antioxidant activity by reducing power of the extracts, DPPH method and Hydrogen peroxide method were evaluated. All the extracts possess good antioxidant activity and the activity decreased in the order of *Emblica officinalis, Saropus androgynous, Phyllanthus niruri,* because of the presence of chemical compounds present in the extracts.

Keywords: *Phyllanthus niruri, Emblica officinalis*, *Saropus androgynous,* Antioxidant Activity.

1. INTRODUCTION

Free radicals such as Reactive Oxygen Species (ROS) in living organisms are known to induce oxidative damage of DNA and Cellular components leading to cancer related mutations. Substances which scavenge free radicals play an important role in the prevention of free radical inducing mechanism in human body. The intake of vegetables and fruits give positive reduced cardiovascular disease and certain cancers. Along with other antioxidant components, polyphenols (e.g. flavanoids) present in fruits and vegetables have been reported to be potential candidates in lowering cardiovascular diseases.The protective effects could be due to their properties as free radical scavengers, hydrogen donating compounds, singlet oxygen quenchers and or metal ion chelators. Antioxidants offer resistance against oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and by other mechanism and thus prevent disease.

*"**Phyllanthus emblica**"* **-** Indian gooseberry

It is also known as *Emblica officinalis,* one of the most frequently used ayurvedic medicine (Chopra, R. N, Nayar S. L, I. C.1956 Glossary of Indian medicinal plants). PE (Amla) is a natural source of vitamin C. The Ascorbic acid present in one fruit is equivalent to that present in two oranges. Its fruit is acrid cooling, refrigerant diuretic, and laxative. The fruits are also used for anemia, hepatopathy, jaundice, diarrhea, hemorrhages, leucorrhoea, cardiac disorder,

cough, bronchitis and grayness of hair (Basa S. C, Srinivasulu C. 1987 *Indian Journal of Natural products).*

"***Sauropus androgynous"***
Sauropus androgynus (SA) of family Euphorbiaceae is widely used as tonic, antioxidant, and febrifuge. The leaves are used as antitussive, tonic and soothing lungs, febrifugal and relieve internal fever (The Ayurvedic Formulary of India 1978). The dark-green leaves has a valuable blood building element, cell rejuvenator and for regular bowel elimination. It is a very good source of potassium, a mineral that has many health benefits.The young shoots, leaves, flowers and fruits can be eaten as vegetables, raw or cooked. The dried crushed root is used medicinally for headache and urinary problems (Dictionary of Indian medicinal plants, 1988). The leaves are thought to stimulate milk production. Dietary antioxidant present in vegetable and fruit can be used against oxidative damage and chronic disease such as cancer. In Malaysia, Taiwan the leaves are eaten either raw or cooked for the purpose of weight reduction.

"***Phyllanthus niruri"***
Originated in India, usually growing as a winter weed throughout the hotter parts (The complete encyclopedia of herbs 2006 by Nico vermeulen). It is a small annual herb grows up to 30-60cm. PN has shown clinical efficiency in viral Hepatitis B .It is known for its liver healing properties and used in Chinese medicine for treatment of liver disease. Its root, leaves, fruits, milky juice and whole plants are used as medicine (Indian Ministry of Health and Family Planning, The Ayurvedic Formulary of India 1978). In Ayurveda, Unani and Siddha systems of medicine it is mostly used for treatment of many diseases like bronchitis, asthma, leprosy, urinary discharge, stomachic, anuria wound etc. As far as our literature survey could ascertain, only few information was available on the *in-vitro* antioxidant activities and the elimination of Vitamin C in these plants.
Therefore the aim of the study was to investigate the antioxidant status by invitro method, the amount of Vitamin C present and HPTLC fingerprint analysis of the above mentioned plants.

2. MATERIALS AND METHODS

2.1. Chemicals

1, 1-dipheyl-2-picryl hydrazyl (DPPH), Ascorbic acid were purchased from Sigma-Aldrich,India. Hydrogen peroxide, trichloroacetic acid, ferric chloride, potassium dihydrogen phosphate, sodium hydroxide, potassium ferricyanide were purchased from Merck,Mumbai,India. Solvents and all the reagents used were of analytical grade.

2.2. Plant materials

Phyllanthus niruri, *Saropus androgynous* (aerial parts) and *Phyllanthus emblica* (fruit) were collected near Kuzhithurai, Kanyakumari Dist, Tamilnadu, India during February, March of 2010 and authenticated in the Department of Botany, Karpagam University, and Coimbatore. The plants were cleaned and shade dried separately. Then powdered and passed through mesh size 80 and stored in an air tight container.

2.3. Preparation of extract

Each (500g) powdered form of the three drugs were extracted with methanol by cold maceration method for 7 days. Then the extracts were filtered and the last traces of the solvent were evaporated under reduced pressure in a rotary evaporator. The yield of the dry extracts were calculated.

2.4. Determination of ash values

About 2 g accurately weighed powdered drug from the three samples were incinerated in a silica crucible at a temperature not exceeding 450^{o}C for 4 hours in a muffle furnace until free from carbon. It was then cooled and weighed. The % w/w of ash with reference to the air-dried drug was calculated. The acid insoluble ash, water soluble ash and sulphated ash was done according to the standard procedure *(Practical Pharmacognosy, Dr C.K.Kokate, 4th edition 1994).*Average of the triplicate values were calculated and mentioned in the (Table No.1).

2.5. Determination of extractive value

Accurately weighed 5 g of air-dried powdered drug was macerated with 100 ml of 90% alcohol of the specified strength in a closed flask for 24 h, shaken frequently during first 6 h and allowed to stand for 18 h. It was then filtered rapidly, taking precautions against loss of the solvent and 25 ml of the filtrate were evaporated to dryness in a tared flat-bottomed shallow dish and dried at 100^{o}C to constant weight. The % w/w of alcohol soluble extractive value was

calculated with reference to the air-dried drug .The same procedure was repeated with different solvents like chloroform, petroleum ether, benzene and water according to the standard procedure *(Practical Pharmacognosy, Dr C.K. Kokate, 4th edition1994)*. The values are mentioned in the (Table No.2)

2.6. Preliminary phytochemical analysis

The extracts prepared in different solvents were taken and standard methods *(Practical Pharmacognosy, Dr C.K.Kokate,4th edition1994)* were used to detect the nature of phytoconstituents present in them .

2.7 Crude Fibre content determination

Accurately weighed 3gm of powdered drug from PN, PE, and SA were extracted with petroleum ether at room temperature and filter dried. From that 2gm of each drug was weighed and boiled with 200ml of sulphuric acid for 30min. Filter through muslin cloth, wash with boiled water filter, again boiled with 200ml of sodium hydroxide solution for 30mts.Then filter and washed with 25ml of 1.25% sulphuric acid, 50ml of boiled water and last alcohol. After filtration Dry the residue at 130^0c for 2hrs and incinerate the residue for 30min then weighed and calculated. The results are given in the (Table.No.3)

2.8. Vitamin C Determination

This method determines the vitamin C concentration in a solution by a redox titration using iodine. Vitamin C, more properly called ascorbic acid, is an essential antioxidant needed by the human body. As the iodine is added during the titration, the ascorbic acid is oxidised to dehydroascorbic acid, while the iodine is reduced to iodide ions.

ascorbic acid + I2 → 2 I− + dehydroascorbic acid

Due to this reaction, the iodine formed is immediately reduced to iodide as long as there is any ascorbic acid present. Once all the ascorbic acid has been oxidised, the excess iodine is free to react with the starch indicator, forming the blue-black starch-iodine complex.This is the end point of the titration. Standared solution of Vitamin C tablet is dissolved in 200ml of distilled water. The freshly collected drugs are cleaned and cut into small pieces and grinded well with addition of little water,and collect 25 ml of fresh juice. Decanted the liquid into volumetric flask, strain the ground drug rinsing the pulp with 10ml water and collect all filtrate into flask. Make up to 100ml with distilled water.

Pipette out 20 ml of the liquid sample into 250ml conical flask and add about 150ml distilled water and1ml starch indicator solution. Titrate the solution with 0.005mol iodine solution. End point is the permanent trace of dark blue-black colour due to starch iodine complex. Repeat the titration to obtain concordant value. The results are given in the table no.3.

2.9. DPPH radical scavenging activity

The free radical scavenging activity was measured by the decrease in absorbance of methanolic solution of DPPH (A. Kumaran, R.Joel science direct.LWt40 (2007), 344-352). A stock solution of DPPH ($33mgL^{-1}$) was prepared in methanol and 5ml of this stock solution was added to 1ml of the PN,PE,SA,extract solutions at different concentrations (25,50,75,100,150, 200,250,2500ug/ml^{-1}).After30min, absorbance was measured at 517nm and compared with the standard ascorbic acid (10-50ugml^{-1}) pH 7.4.Percentage of DPPH scavenging activity of the PN, PE & SA extract and the standard was calculated. The percentage extract of inhibition was calculated by the formula [(Ao-A1)/Ao] x100, when Ao is the absorbance of the control & A1 is the absorbance of the extract/standard.

2.10. Reducing power determination

The reducing power of the extracts was determined according to the Oyaizu method (Oyaizu, 1986). Different concentrations of extracts/standard (50-250mgm/ml) in methanol were mixed with phosphate buffer (PH 6.6) and incubated with (2.5ml) of potassium ferricyanide solution (1%w/v) at 50^oC for 20 min.After mixing, the mixture was incubated at 50^oC for 20 min.The 2.5 ml of trichloroacetic acid was added to the mixture and which was then centrifuged for 10 min.The supernatant (2.5ml) was mixed with distilled water (2.5ml) and ferric chloride (0.5ml) and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicates increased reducing power.

2.11. Scavenging of hydrogen peroxide

The ability of three extracts to scavenge hydrogen peroxide was determined according to the method of (Ruch, Cheng, and Klauning, 1989). A solution of hydrogen peroxide (2mol/l) was prepared in phosphate buffer (PH 7.4). Hydrogen peroxide concentration was determined by spectrometrically absorbance at 230nm.Extracts were prepared at the

concentration of 50-250mgm/ml and added to the hydrogen peroxide solution (0.6ml). Blank solution contains phosphate buffer with without hydrogen peroxide .For each concentration a separate blank sample was used for background subtraction. The % of inhibition activity was calculated from the formula $[(A_0 – A_1)/A_0]$ X 100.Where A_0 is the absorbance of the control and $A_1$is the absorbance of extract/standard.

Statistical analysis
Values were represented as mean ± SD of three parallel measurements and data were analysed using the t-test.

2.12. High performance thin layer chromatography
The different methanolic extracts were subjected to preliminary phytochemical screening to identify the presence of various phytoconstituents present in the extract. Commercially available precoated HPTLC plates of Silica gel 60 F_{254} (Merck, India) were used for the study. The solutions of the three extracts were applied on the respective HPTLC plates using Linomat IV applicator. The plates were dried after application. Twenty microlitres of methanolic fraction of the extracts were spotted in the form of a band and also ascorbic acid and epicatechin also spoted using Linomat IV Sample Applicator (Camag, Switzerland).TLC pattern was developed using Toluene: ethyleacetete (9:1). Then the plates were scanned in Camag Scanner at a wavelength of 365nm. Peak areas and peak heights were recorded from which percentage of separated compounds were determined.

3. RESULTS AND DISCUSSION
(A)Extractive value (Table 1)

(B) Ash values
The ash values of the three powdered drug samples S1, S2 & S3 were calculated with reference to the weight of air dried powdered drug and the values are given in (Table 2)

(C) Fibre content and Vitamin C Estimation
The Fibre content of three powdered drug samples S1, S2, & S3 were calculated and the values are given in Table No.3.

D) Phytochemical test
Qualitative phytochemical analysis of *Phyllanthus emblica, Phyllanthus niruri* and *Sauropus androgynus* extracts showed the presence of alkaloids, carbohydrates, proteins, free amino acids, tannins & phenolic compounds, steroidal glycosides, and flavanoids.

E) Anti oxidant activity
Free radicals, mainly the Reaction Oxygen Species (ROS) are involved in initiation, promotion and progression of carcinogenesis. Reactive Oxygen Species induce oxidative damage of DNA and other cellular components leading to cancer related mutations.Consequently antioxidants play an important role in the protection of human body against damage by reactive oxygen species and also the intakes of natural antioxidants has been associated with reduced risks of cancer and other diseases related with oxidative damages.
The three plants are possessing radical scavenging activity which is mostly related to the phenolic compound and the phenolic hydroxyl group. The concentration of hydrogen peroxide in water varies according to the phenolic compound. Since phenolic compounds present in the extract are good electron donors, they may accelerate the conversion of H_2O_2-H_20.Reactive Oxygen Species (ROS) including free radical such as super oxide anion radicals (O_2^-), hydroxyl radicals (OH) non free radicals such as H_2O_2, Singlet oxygen (O_2) along with various forms of active oxygen are involved in various physiochemical processes in the body and ageing.
Hydrogen peroxide is mainly produced by enzymatic reaction. These enzymes are located in microsomes, peroxysomes and mitochondria. In plant and animals cells super oxide dismutase is able to produce hydrogen peroxide by dismutation of oxygen, thus contributing to the lowering of oxidative reactions. The natural combination of dismutase and catalase contributes to remove hydrogen peroxide and thus has a true cellular anti oxidant activity. Hydrogen peroxide is also able to diffuse easily through cell membrane. The generation of hydrogen peroxide by activated phagocytes is known to play an important part in the killing of several bacterial and fungal strains. The ability of the plant extracts to scavenge hydrogen peroxide is followed by decay in hydrogen peroxide concentration.$O^{2-} + H_2O_2 \rightarrow OH^- + OH^+ + O_2$.

Hydrogen peroxide method
The IC50values and scavenging ability of three plants methanolic extract with hydrogen

peroxide is shown in table no.4 compared with the standard ascorbic acid and epicatechin. It is noticed that all the extracts are capable of scavenging hydrogen peroxide in an amount dependent manner.

Reducing power determination

The results of reducing power determination of the three species were determined and their I.C.50 values are shown in the table 4

DPPH method

The antioxidant properties of the three species are given in the table no. 4. The results of free radical scavenging activity of mehanolic extract of the three plants and the positive control ascorbic acid and epicatechin in DPPH free radical system were shown in the Graph 2.

Table 1: The extractive values of the three drugs

S. No.	Solvent	Extraction value %w/w		
		S1	S2	S3
1	Methanol	22	15	24.1
2	Chloroform	1.2	0.7	9.67
3	Benzene	13.7	14.6	17.4
4	Petroleum Ether	0.4	2.9	13.3
5	Water	4.7	5.1	12.6

S1- *Phyllanthus emblica* S2- *Phyllanthus Niruri* S3- *Sauropus androgynous*

Table 2: ash values of the three powdered drug samples

S. NO.	ASH VALUES	Value in percentage W/W %		
		S1	S2	S3
1	Total Ash	4.8	5.9	4.5
2	Acid insoluble ash	2.5	1.1	1.2
3	Water insoluble Ash	1.3	1.4	1.3
4	Sulphated Ash	3.2	3.2	2.4

S1- *Phyllanthus emblica* S2- *Phyllanthus niruri* S3- *Sauropus androgynus*

Table 3: Fibre content of three powdered drug samples

S. No.	Sample name	Fibre content w/w %	Vitamin C
1	Phylanthus emblica	1.102	0.67gm/ 25ml
2	Phylanthus niruri	1.415	0.25 gm/25ml
3	Saropus androgynus	1.024	0.39 gm/25ml

Table 4: Antioxidant activity of three species and their I.C.50 values

S. No.	Samples	DPPH mehtod	Hydrogen peroxide	Reducing power
1	*Phyllathus emblica*	110µg/ml	61µg/ml	93µg/ml
2	*Phyllanthus niruri*	180µg/ml	60µg/ml	105µg/ml
3	*Sauropus androgynus*	235µg/ml	63µg/ml	95µg/ml
4	*Ascorbic acid*	90µg/ml	35µg/ml	43µg/ml
5	*Epicatechine*	82µg/ml	28µg/ml	56µg/ml

Values represent the mean ± SD SEM: Number of readings in each group = 3.

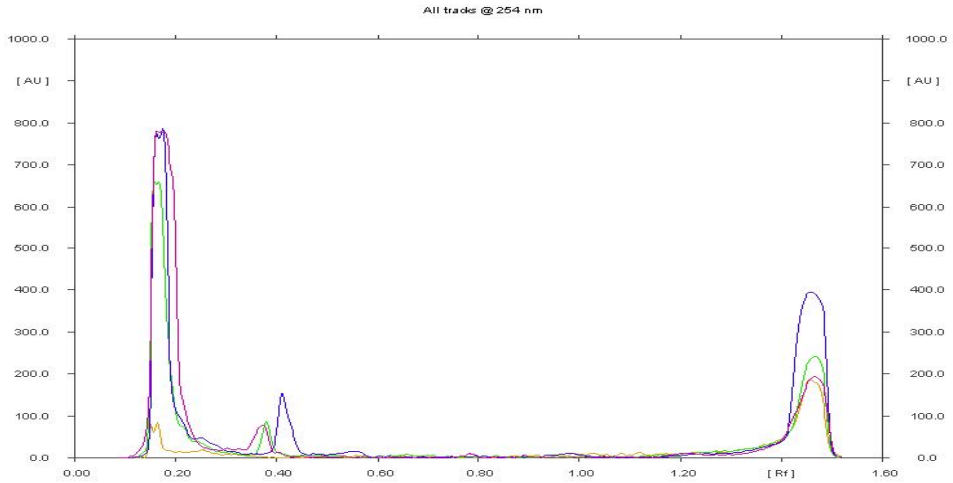
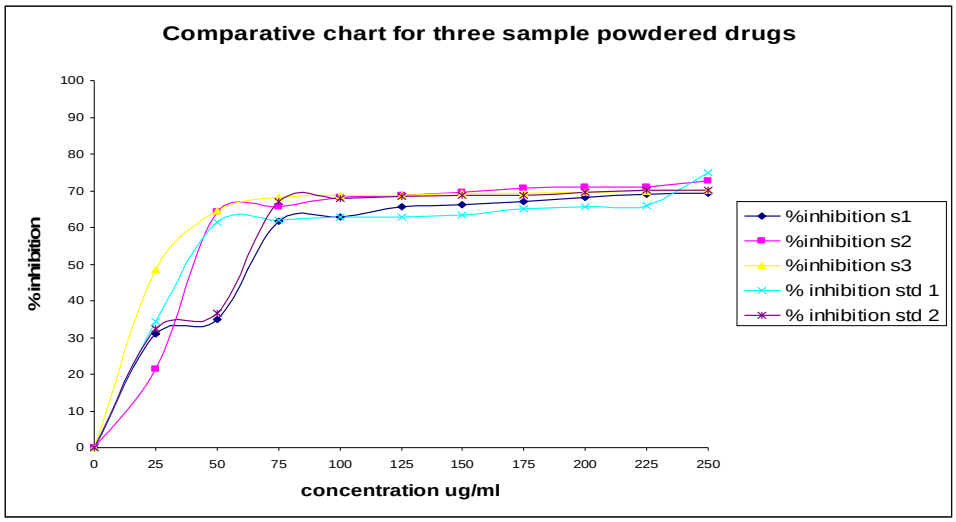


Table 5: HPTLC ANALYSIS

S. No.	Particulars	No of Peaks	RF Value	Area	Area %
1	Alcoholic extract of *Phyllanthus emblica*	5	0.10, **0.34**,0.75,0.6,0.32	6271.7	23.97%
2	Alcoholic extract of *Phyllanthus niruri*	5	0.12,0.24,**0.37**,0.50,0.29	11932.4	43.13%
3	Alcoholic extract of*Saurapus androgynous*	3	0.11,0.34, **0.34**	6941.9	35.89%
4	Ascorbic acid	1	0**.37**	5253.1	77.17%
5	Epicatechin	1	0.12	6019.1	100%



Graph 2: The antioxidant activity of three species with standards by DPPH method

CONCLUSION

The present study aimed to do a comparative study about the pharmacognostical, Physicochemical, phytochemical and their antioxidant activity of three plants (P.N.,P.E.,S.A.) from Euphorbiaceae family . The antioxidant activity of polyphenols is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching oxygen, or decomposing peroxides. Indeed the antioxidant activity of the plants extracts is highly correlated with the total phenol content. The crude mathanolic extract of PE, PN, SA, exhibited a significant antioxidant

activity which may be relevant in the treatment of oxidative stress. The antioxidant activity was measured as free radical scavenging activity DPPH method, Reducing power determination method, Hydrogen peroxide method. All the methods show good response due to the presence of phenolic compounds and flavonoids in three species. Among all the three extracts tested, showed highest antioxidant activity with IC 50 value in the order of PE > PN > SA when compared to the standards. The result reveals that all the extracts have the scavenging character in accordance with the standards. The further work has been developed for the isolation of particular phenolic compound for this activity, and also can be used for the new formulation development.It is well known that Phenols and vitamin Cv and have beneficial effect on health .So addition of crude extracts to food products would be interesting for functional foods preparation like juices or jams, could be a valuable tool for fortification of foods with Phenolic compounds and vitamin C as well as for stabilization of anthocyanins.

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