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ANTIOXIDANT EFFICACY AND EVALUATION OF CRUDE DRUG PARAMETERS OF *GLYCOSMIS PENTAPHYLLA* (RETZ.) DC

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ABSTRACT: The present work has been done with an objective to analyze the crude drug parametrs responsible for tenable antioxidant effect of leaf, stem and root of *Glycosmis pentaphylla* (Retz.) DC. Fresh leaf, stem and root of *Glycosmis pentaphylla* were extracted with ethanol (EEGPle, EEGPst, EEGPro). The antioxidant profiling was done through different methods like 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, 2,2-azino-bis-3-ethyl benzothiazoline acid (ABTS) free radical scavenging assay and nitric oxide (NO) free radical scavenging assay. Crude drug parameters were focussed mainly on micrometric properties so as to determine the flow properties when consumed for medicinal purposes. A significant antioxidant activity was revealed by the EEGPle [EC_{50}: (29.04±0.008) µg/mL], compared to EEGPst and EEGPro in DPPH assay. The ranking order for ABTS was EEGPle > EEGPst > EEGPro. The EC_{50} value of NO of the EEGPle, EEGPst and EEGPro were (263.14±0.05) µg/mL, (312.62±0.07) µg/mL and (384.62±0.006)µg/mL respectively. Quantity of TFC and TPC were highest in EEGPle (112.96±3.89 mg QRE/g and 96.6±1.08 mg GAE/g extract) rather than EEGPst and EEGPro extracts. The present work suggests that EEGPle has a significantly higher antioxidant property than EEGPst and EEGPro. These extract can help in prevent or slow down the occurrence of different diseases related to oxidative stress. But, a detailed analysis of these extracts is required to determine the presence of promising compound(s) responsible for their antioxidant activity. Better quality control parameters in pharmaceutical industries helps to prevent the adulteration, if any. Hence, this work will help in identification and quality control of *G. pentaphylla* as a medicinal material.

INTRODUCTION: Ever since time immemorial, human beings have looked for drugs from nature to cure diseases. Man started using medicinal plants since 3000 BC [1]. Since that time, man started doing trial and error experiments to expand their knowledge on the usage of medicinal plants. Simultaneously, they have started preserving plants in dried form or so and also began cultivations too. For example, *Papaver somniferum* (poppy) was known to cultivate in lower Mesopotamia [2], and *Cananbis sativa* (cannabis) was cultivated in Europe [3]. More than 50,000 species of plants are known to have medicinal properties and are used in pharmaceutical and cosmetic industries. The active principles present in the leaf, stem, root, fruit, flower or in fact the whole plant can have direct or indirect therapeutic effects and are used as medical agents [4].

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Ensuring the safety, efficacy and quality of medical plants and herbal drugs is a key issue in industrialized and developing countries. Hence, there is a need of standardization and evaluation of the active components present in plants so that, it can help in the emergence of a new millennia of preventive medicine to treat human diseases in future.

Oxidative stress is a phenomenon which is caused by an imbalance between the production and accumulation of reactive oxygen species (ROS) (like superoxide radicals, hydrogen peroxide, hydroxyl radicals *etc*.) in cells and tissues and the ability of biological system to detoxify these reactive products. On accumulation, these ROS can harm important cellular structure of the body like proteins, lipids and nucleic acids [5]. This can further lead to the onset/ progression of several diseases like cancer, atherosclerosis, diabetes, metabolic disorders and cardiovascular diseases [6].

Most of the organisms are capable of combating oxidative stress damage through enzymes like catalase, superoxide dismutase, antioxidant compounds like ascorbic acid, tocopherol, phenols, flavonoids, glutathione and so on [7]. The emergence of allopathic drugs are also well appreciated in terminating ROS, but they are at times reported to cause severe side effects like gastrointestinal disorders, memory loss *etc*.

In this scenario, consumption of diet rich in natural antioxidants derived from medicinal plants gathers attention. These herbal drugs can ameliorate oxidative damage and can be useful to treat the plethora of oxidative stress related diseases.

The plant *Glycosmis pentaphylla* (Retz.) DC. (*G. pentaphylla*) of the citrus family, Rutaceae is an evergreen shrub or small tree that grows up to 5 metres tall. The plant is instinctive to China, India, Sri Lanka, Thailand, Cambodia, Vietnam, Malaysia, Indonesia and Philippines. The detailed description of plant is available elsewhere [8]. The fruit of the plant is edible and the juice of leaves are used to treat diarrhoea, coughs, rheumatism, anaemia and jaundice [9]. Several authors investigated the plant for anti-inflammatory efficacy [10], hepatoprotective activity [11] antimicrobial effect [12] and antipyretic potential [13].

Previous research have revealed that *G. pentaphylla* possess antioxidant activity in its leaves [14]. But there are no reports showing comparison of the free radical scavenging activity of the plant in its leaf, stem and root. The plant is of great importance yet there isn't any extensive and thorough pharmacognostic data available on its structural anatomy and physicochemical standards, as it is required for the identification and quality standardization of the plant. Therefore, an attempt to explore the phytochemical composition and antioxidant activity of ethanolic extract of *G. pentaphylla* leaf (EEGPle), stem (EEGPst) and root (EEGPro) is made.

MATERIALS AND METHODS:

Chemicals: Ascorbic acid (AA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis-3-ethyl benzothiazoline acid (ABTS) nitric oxide (NO), pottsium ferricyanide, ammonium molybdate and trichloroacetic acid, were acquired from Sigma-Aldrich, USA and other remaining chemicals were used of analytical grade unless otherwise specified.

Plant Materials: Fresh leaf, stem and root (about 5 kg) of *G. pentaphylla* were collected from Thodupuzha (Latitude: 9.8959°N; Longitude: 76.7184°E) of Kerala, India during April, 2016 to October, 2018. The leaves, stem and roots were thoroughly washed to remove foreign matters, and then shade dried for 2 weeks. Later, the identification was done as *G. pentaphylla* (Accession number KUBH 6073) by an expert taxonomist from Department of Botany, University of Kerala, India.

Collection and Authentication of Plant Materials: The leaf, stem and root of *G. pentaphylla* (about 2 kg each) were seperated manually, followed by shade dried. Dried leaf, stem and root were grounded with the help of a mechanical grinder into coarse powder. The generated powders (about 300 g each) were preserved in airtight containers and placed in a cool, dry and dark place until extraction.

Preparation of Plant Extract: Based on literature review, it was found that, ethanol possess higher extraction efficiency due to its polarity [15]. About 200 g individual powdered sample was taken in clean, flat-bottomed amber colored glass container

and soaked in 700ml of 95% ethanol. The container with this contents was sealed and kept for several days accompanying occasional shaking. The whole mixtures then underwent coarse filtration by pieces of cotton. Thereafter, the mixture was filtered through filter (Whatman no. 1) paper and the solvent was made to evaporate under reduced pressure with the help of a rotary evaporator at 50 ^{o}C to yield crude extracts. (*i.e.* 10.75 g for EEGPle, 6.18 g for EEGPst and 8.25 g for EEGPro). The crude ethanolic extracts thus obtained were kept at 4 ^{o}C for further studies.

Micrometric Evaluation of Dried Plant Powder: The physical characteristic of dried plant powder was evaluated as per pharmacopoeial procedures. The physical characteristics like Bulk density, Tap density, Angle of repose, Hausner's ratio and Carr's index indicates the flow properties as well as interparticulate resistance between powders. The information collected from this evaluation was crucial to avoid ambiguous predictions of stability or solubility of crude drug. The micrometric properties help to characterize and standardize the pre-formulation properties of the herbal drug powder, in order to determine its suitability for formulation into solid dosage forms.

Bulk Density: [16] It is the ratio of given mass of powder and its bulk volume. About 50 g of dried plant powder was transferred to a graduated cylinder with the aid of a funnel. The volume (initial) formed after the adding of powder was noted. The ratio of weight to the volume it occupied was calculated as:

$$\text{Bulk Density} = W / (V_O)\ \text{g/ml}$$

Where W = mass of the powder, V_O = untapped volume

Tapped Density: [17] About 25 g of dried plant powder was transferred to a graduated cylinder and tapped it for a specific number of times. The initial volume was noted. The cylinder was continuously tapped for a period of 20 min. The final volume noted. The tapped density is determined as the ratio of mass of the powder to the tapped volume.

$$\text{Tapped volume} = W / (V_f)\ \text{g/ml}$$

Where W = mass of the powder, V_f = tapped volume (density)

Angle of Repose: [18] Angle of repose is used as indirect method of quantifying powder flowability. As a general rule, powder with angle of repose greater than 50 degree has unsatisfactory flow properties, whereas minimal angle close to 25 degrees correspond to very better flow properties. Angle of repose is the maximum angle possible between the surface of a pile of the powder and the horizontal plane. A glass funnel was held in place with a clamp on a ring support over a glass plate.

About 50 g of powder was transferred in to the funnel. As the thumb was removed, the lab-jack was adjusted so as to lower the plate and maintain about a 6.4 mm gap between the bottom of the funnel stem and the top of the powder pile [19]. When the powder was emptied from the funnel, the angle of the heap to the horizontal plane was measured with the protractor and calculated by following formula.

$$\tan \theta = h / r \text{ or } \theta = \tan^{-1} h / r$$

Where, h = height of pile, θ = angle of repose, r = radius of the base of the pile

Carr's Compressibility Index: The Carr's compressibility index is an indication of the compressibility of the powder. The value gives an indication on the flowability of the powder. The more compressible the material the less flowable it will be. A powder with a compressibility index less than 20% is considered to have a good flowability [20]. Based on the apparent bulk density and tapped density, the percentage of Carr's compressibility of the powder was calculated as follows.

$$\text{Percentage compressibility} = (V_O - V_f) / V_O \times 100$$

Where VO = bulk density and Vf = tapped density

Hausner's Ratio (H): It indicates flow properties of drug powder [21]. The ratio of tapped density to the bulk density of the powder is called Hausner's ratio. This ratio is a useful measure of cohesion reflecting particle friction. With a Hausner's ratio higher than 1.4, the powder is considered a cohesive difficult to fluidize powder. Ratios lower than 1.25 characterizes as free-flowing powder [22, 23].

$$\text{Hausner's ratio (H)} = \text{tapped density} \times 100 / \text{bulk density}$$

Antioxidant Activity: The samples were dissolved in methanol (95% v/v) to get 1 mg/mL concentration and utilized for antioxidant assays.

DPPH Radical Scavenging Assay: The radical scavenging activity of leaf, stem and root extracts of *G. pentaphylla* was determined by using DPPH assay according to Chang [25] with minor modifications. The principle of this assay is that 1, 1-diphenyl-2-picryl hydrazyl is a stable free radical with red colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or was measured at 517 nm. Ascorbic acid (10 mg/ mL DMSO) was used as a reference. The DPPH solution was prepared in methanol (95%) toward getting a concentration of 240 µg/mL. The stock solution of 1 mg/ mL was prepared by mixing *G. pentaphylla* crude extracts with 95% methanol solution. The stock solution was used for the preparation of test solution through dilution with methanol to get the appropriate concentrations (25, 50, 100 and 200 µg/mL). A standard solution of AA was prepared in the same way as described above. A recently prepared DPPH solution (4 mL) was mixed in each of the test tubes having 100 µL extracts. The mixture was vigorously shaken and placed aside for the 30 min reaction period at room temperature in a dark room. After incubation, the absorbance of the mixture was recorded by UV spectrophotometer at 517 nm against methanol as a blank and experimental procedure was repeated for three times. The control used for the study was DPPH solution without sample solution. The percentage scavenging activity at different concentrations was determined by the following formula;

$$\text{DPPH radical scavenging activity (\%)} = [1 - (A_1/A_0)] \times 100$$

Where, A_0 is the absorbance of control reaction, A_1 is the absorbance of test compound/standard solution.

ABTS Radical Scavenging Assay: The ABTS radical cation method [26] was modified to evaluate the free radical scavenging effect of leaf, stem and root extracts of *G. pentaphylla*. The ABTS reagent was prepared by mixing 5 mL of 7 mM ABTS with 88 µL of 140 mM potassium persulfate. The mixture was then kept in the dark at room temperature for 16 h to allow free radical generation and was then diluted with water (1:44 v/v). To determine the scavenging activity, 100 µL ABTS reagent was mixed with 100 µL of each extract and was incubated at room temperature for 30 min. The stock solution was used for the preparation of test solution through dilution with methanol to get the appropriate concentrations (25, 50, 100 and 200 µg/mL). A standard solution of AA was prepared in the same way as described above. After incubation, the absorbance was measured 734 nm using a UV spectrophotometer against methanol as a blank and experimental procedure was repeated three times. The control used for the study was ABTS solution without sample solution. The ABTS scavenging effect was measured using the following formula:

$$\text{ABTS radical scavenging activity (\%)} = [1 - (A_1/A_0)] \times 100$$

Where, A_0 is the absorbance of control reaction, A_1 is the absorbance of test compound/standard solution.

NO Scavenging Assay: NO scavenging activity in leaf, stem and root of *G. pentaphylla* was done by the procedure of Garrat [27] with minor modifications. The extracts were prepared from a 10 mg/mL ethanol crude extract. These were then serially diluted with distilled water to make concentrations of 25, 50, 100 and 200 µg/mL and the standard gallic acid. These were stored at 4 °C for later use. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene-diaminedihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 mL of the different concentrations of the ethanol extracts and incubated at 25 °C for 180 min. The extracts were mixed separately with an equal volume of freshly prepared Griess reagent. Control samples without the extracts but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. The colour tubes contained ethanol extracts at the same concentrations with no sodium nitroprusside. A volume of 150 µL of the reaction mixture was transferred to a cuvette and the absorbance was measured at 546 nm using a UV spectrophotometer.

Gallic acid was used as the positive control. The percentage nitrite radical scavenging activity of the ethanol extracts were calculated using the following formula:

$$\text{NO radical scavenging activity (\%)} = [1 - (A_1/A_0)] \times 100$$

Where, A_0 is the absorbance of control reaction, A_1 is the absorbance of test compound/standard solution.

Total Phenolic Content (TPC): The TPC was estimated according to Cheung *et al*. [28, 29] The crude extracts of *G. pentaphylla* were mixed with methanol (95%) for preparation of the stock solution (1 mg/mL). A standard, GA was also mixed with 95% methanol to prepare the 1 mg/mL concentration standard solution. For this test, 1 mL of crude extract with 1000 µg/mL concentration was mixed along with 1 mL Folin-Ciocalteu's reagent, 5 min later 10 mL volume of sodium carbonate (7%) solution was added to the mixture, and then deionized distilled water (13 mL) was added and thoroughly mixed. This mixture was kept for 90 min in the dark at 23 ºC and then the absorbance was recorded at 750 nm by UV spectrophotometer. Standard curve for estimation of TPC was prepared using GA standard solution (*i.e.* 6.25 µg/mL to 200 µg/mL) using the similar procedure as described earlier. The TPCs were expressed as mg of gallic acid equivalents (GAE) per g of the dried sample.

Total Flavonoid Content (TFC): The TFC was estimated by method described by Park *et al*. [30] The stock solution was prepared as mentioned in TPC. Similarly, the standard solution of QT was prepared through mixing it with 95% methanol (*i.e.* 1 mg/L). To estimate the TFC, 0.3 mL of the crude extract (1000 µg/mL), 3.4 mL of methanol (30%), 0.15 mL of 0.5 moL/L sodium nitrate, and 0.15 mL of 0.3 moL/L aluminum chloride were mixed. Then after 5 min, 1 mL of 1 moL/L sodium hydroxide was supplemented. The obtained solution was thoroughly mixed and absorbance was recorded at 506 nm against the reagent blank. TFCs were expressed as mg of quercetin equivalents (QRE) per g of the dried sample.

Statistical Analysis: All experimental results are expressed as mean ± standard error (SD), and data were analysed by one-way analysis of variance ($P < 0.001$) using SPSS software (ver. 21.0; SPSS Inc., Chicago, IL, USA).

RESULTS:

Determination of Crude Drug Parameters: The micrometric properties also showed good flow properties for the leaf powder **Table 9**. The leaf powder of *G. pentaphylla* had Hausner's ratio of 2.10 ± 0.02 whereas the stem and root powder showed 1.55 ± 0.07 and 1.65 ± 0.07 respectively. Carr's compressibility index of leaf, stem and root were 9.38 ± 1.02%, 7.27 ± 0.18% and 6.41 ± 0.14% respectively. The leaf powder showed a good flow rate of 2.56, whereas the flow rate of stem and root powder were 2.01 and 2.89 g s^{-1} respectively. Angle of repose for leaf, stem and root powder was 20.3°, 22.1° and 23.2° respectively.

TABLE 1: MICROMETRIC PARAMETERS

Parameter	Leaf	Stem	Root
Bulk density	0.22±0.03 (ml/g)	0.19±0.02 (ml/g)	0.20±0.04 (ml/g)
Tap density	0.28±0.07 (ml/g)	0.16±0.09 (ml/g)	0.17±0.03 (ml/g)
Hausner's Ratio	2.05±0.02	1.55±0.07	1.65±0.07
Carr's compressibility index	9.38±1.02%	7.27±0.18%	6.41±0.14%
Flow rate	2.56 (g/s)	2.01 (g/s)	2.89 (g/s)
Angle of Repose	20.3º	22.1º	23.2º

Estimation of DPPH: The DDPH free radical scavenging assay of ethanolic extract of leaf, stem and root of *G. pentaphylla* is depicted in **Table 2**. The scavenging activity is of the following order: AA >EEGPle>EEGPst>EEGPro. It was found that, the extract possessed a dose-dependent activity which indicates that, the as the concentration of the extract increased, DPPH free radical scavenging activity also increased. The EC_{50} value of scavenging DPPH radicals for AA<EEGPle, EEGPst and EEGPro were also shown in **Table 2**. On comparison with the EC_{50} value AA, EEGPle was significantly higher ($P<0.05$). Thus, the present result reveals that, among the three extracts, EEGPle exerted a 74.2% scavenging activity at 200 µg/mL concentration.

TABLE 2: DPPH SCAVENGING ACTIVITY OF *G. PENTAPHYLLA* LEAF, STEM AND ROOT EXTRACTS

Standard/Extract	DPPH Scavenging (%)				EC_{50} value for DPPH radical
	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	
Ascorbic acid	55.8±0.58	62.2±0.86	75.4±0.74	77.8±0.58	22.33±0.009
EEGPle	43.0±0.70	53.0±0.70	71.4±0.50	74.2±0.66	29.04±0.008
EEGPst	19.4±0.50	27.0±0.70	37.8±0.58	43.0±0.70	222.23±0.007
EEGPro	11.6±0.50	22.8±0.58	31.2±0.58	37.0±0.70	1081.05±0.006
Treatment df (n-1)	1236.814***	718.224***	1371.547***	989.12***	3870.906***

The representative experiment is a mean standard error followed by different superscript lowercase letters indicate significant difference between each parameter as evaluated by Duncan's Multiple Range Test. f value significant at *P≤0.001 level, NS-non-significant.

Estimation of ABTS: The ABTS free radical scavenging assay of ethanolic extract of leaf, stem and root of *G. pentaphylla* is depicted in **Table 3**. The scavenging activity is of the following order: AA >EEGPle>EEGPst>EEGPro. It was found that, the extract possessed a dose-dependent activity which indicates that, the as the concentration of the extract increased, ABTS free radical scavenging activity also increased. The EC_{50} value of scavenging ABTS radicals for AA<EEGPle, EEGPst and EEGPro were also shown in **Table 2**. On comparison with the EC_{50} value AA, EEGPle was significantly higher ($P<0.05$). Thus, the present result reveals that, among the three extracts, EEGPle exerted a 67% scavenging activity at 200 µg/mL concentration.

TABLE 3: ABTS SCAVENGING ACTIVITY OF *G. PENTAPHYLLA* LEAF, STEM AND ROOT EXTRACT

Standard/Extract	DPPH Scavenging (%)				EC_{50} value for ABTS radical
	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	
Ascorbic acid	47.4±0.67	55.6±0.50	67.4±0.67	77.0±0.70	26.03±0.006
EEGPle	36.8±0.58	50.0±0.70	57.4±0.50	67.0±0.70	49.02±0.006
EEGPst	22.2±0.58	34.6±0.50	47.0±0.70	57.0±0.70	106.37±0.006
EEGPro	10.4±0.50	18.2±0.37	22.4±0.50	37.6±0.50	263.15±0.007
Treatment df (n-1)	753.752***	973.701***	1008.892***	642.629***	2352.725***

The representative experiment is a mean standard error followed by different superscript lowercase letters indicate significant difference between each parameter as evaluated by Duncan's Multiple Range Test. f value significant at *P≤0.001 level, NS-non-significant.

Estimation of NO: The NO free radical scavenging assay of ethanolic extract of leaf, stem and root of *G. pentaphylla* is depicted in **Table 4**. The scavenging activity is of the following order: AA >EEGPle>EEGPst>EEGPro. It was found that, the extract possessed a dose-dependent activity which indicates that, the as the concentration of the extract increased, NO free radical scavenging activity also increased. The EC_{50} value of scavenging NO radicals for AA<EEGPle, EEGPst and EEGPro were also shown in **Table 2**. On comparison with the EC_{50} value AA, EEGPle was significantly higher ($P<0.05$). Thus, the present result reveals that, among the three extracts, EEGPle exerted a 37.2% scavenging activity at 200 µg/mL concentration.

TABLE 4: NO SCAVENGING ACTIVITY OF *G. PENTAPHYLLA* LEAF, STEM AND ROOT EXTRACT

Standard/Extract	DPPH Scavenging (%)				EC_{50} value for NO radical
	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	
Ascorbic acid	13.0±0.70	20.6±0.50	33.0±0.70	47.4±0.50	212.75±0.004
EEGPle	9.8±0.37	16.8±0.58	27.0±0.70	37.2±0.58	263.14±0.005
EEGPst	7.8±0.37	13.8±0.58	21.4±0.50	32.8±0.66	312.52±0.007
EEGPro	4.6±0.50	10.6±0.50	17.8±0.37	24.6±0.50	384.62±0.006
Treatment df (n-1)	47.795***	60.656***	126.324***	277.538***	1534.085***

The representative experiment is a mean standard error followed by different superscript lowercase letters indicate significant difference between each parameter as evaluated by Duncan's Multiple Range Test. f value significant at *P≤0.001 level, NS-non-significant.

DISCUSSION: Traditional medicine systems mainly relay on natural products. These natural products can range from unicellular to multicellular forms. But among them, plants are in fact the most potential sources of natural antioxidants [31]. Inclusion of fruits and vegetables in diet has

decelerated the occurrence of chronic diseases associated with aging such as cancer, cardiovascular diseases, brain dysfunction and cataract [32, 33]. Also, plants serve as sources for the development of drugs in contemporary medicine. Still, there is a need to determine the safety and stability of plant products before being marketed. Hence, researchers are focusing on the potential of medicinal plants to be used as crude drugs. As part of this, in the present study, the phytoconstituents, and the antioxidant activities of the ethanolic extracts of leaf, stem and root of *G. pentaphylla* were evaluated.

The micrometric properties like bulk density, tap density, angle of repose, Hausner's ratio and Carr's index indicates the flow properties as well as interparticulate resistance between powders [34]. This information predicts the stability and solubility of crude drug. Increase in bulk density reduces paste thickness which is important in preparation of drugs [35]. The ratio of tapped density to the bulk density of the powder is called Hausner's ratio[36]. This ratio is a useful measure of cohesion reflecting particle friction. With a Hausner's ratio higher than 1.4, the powder is considered a cohesive difficult to fluidize powder [37]. Ratios lower than 1.25 characterizes as free-flowing powder. Carr's compressibility index is good if the value ranges between 5% - 15% [38]. If the angle of repose is more than 50°, the powder will not flow satisfactorily and if it is near 25°, the powder will flow easily [39]. The micrometric properties help to characterize and standardize the pre-formulation properties of the herbal drug powder, in order to determine its suitability for formulation into solid dosage forms [40, 41]. The process of standardization can be achieved by stepwise pharmacognostic studies as stated above. Therefore, the result generated from this study would be useful in identification and standardization of the plant material towards quality assurance and also for preparation of a monograph on *G. pentaphylla* plant.

The principle of antioxidant activity is based on the availability of electrons to neutralize free radicals. Performing a single assay to evaluate the antioxidant properties would not give the correct result because the antioxidant activity of plant extract is influenced by many factors, for example, the test system and composition of the extract. Therefore, it is important to carry out more than one type of antioxidant capacity measurement to cover the various mechanisms of antioxidant action [42]. In this study, the antioxidant activity of ethanolic extract of *G. pentaphylla* leaf, stem and root was evaluated by various *in-vitro* methods.

The DPPH are often used to evaluate the ability of antioxidants to scavenge free radicals [43]. In this assay, radicals are reduced to their stable or less reactive derivatives by the antioxidant compounds. In the present investigation, the EEGPle exhibited maximum scavenging activity against DPPH radical than EEGPst and EEGPro in a significant dose-dependent fashion. The present findings are in harmony with reports describing the dose-dependent antioxidant activity [44]. The effect of higher antioxidant activity in the EEGPle could be due to the high percentage of major secondary metabolites as well as other minor components in small quantities or the synergistically actions between the both. From the results, it seems that EEGPle are capable of scavenging the free radical DPPH. Several reports confirm these data [45, 46].

The principle behind the ABTS assay involves the reaction between ABTS and potassium persulphate to produce the ABTS radical cation a blue, green chromogen. In the presence of an antioxidant reductant, the coloured radical is converted back to colourless, the absorbance of which is measured at 734 nm. It is often used in evaluating the total antioxidant power of single compounds and complex mixtures of various plants [47]. The scavenging ability of *G. pentaphylla,* crude extracts on $ABTS^{\bullet+}$ free radical was done using ascorbic acid as the standard. It was found that radical scavenging activity increased as the concentration of crude extracts increased. From the plot of concentration against percentage inhibition, a linear regression analysis was performed to obtain the EC_{50} value. The presence of specific compounds in the EEGPle of *G. pentaphylla* may inhibit the potassium persulfate activity and hence reduced the production of $ABTS^{\bullet+}$. A similar kind of trend could be observed in a study made in the same plant by Gupta N *et al*., [48], where an ethanol extract of *G. pentaphylla* plant showed $ABTS^{\bullet+}$ free radical scavenging activity with EC_{50} value of 26.2 µg/mL compared to an ascorbic acid having an EC_{50} value of 22.8 µg/mL.

Nitric oxide (NO) is an essential bioregulatory molecule required for several physiological processes like neural signal transmission, immune response, control of vasodilation and control of blood pressure [49]. However, the elevation of NO results in several pathological conditions, including cancer. The plant/plant products may have the property to counteract the effect of NO formation and in turn, may be of considerable interest in preventing the ill effects of excessive NO generation *in-vivo*. Incubation of solutions of sodium nitroprusside in phosphate buffer at 25 °C for 30 min resulted in linear time dependent nitrite production, which is reduced by the tested ethanol extract of *G. pentaphylla*. This may be due to the antioxidant principles in the extract, which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite. In order to evaluate the antioxidant potency through NO scavenging by the test samples, the change of optical density of NO was monitored. The EC_{50} values are calculated from the graph using linear regression. The EEGPle significantly inhibited the generation of NO in a concentration-dependent manner as compared to EEGPst and EEGPro. This may be due to the presence of the antioxidant principle present in EEGPle which inhibited the binding of oxygen to nitric oxide. The reports regarding the inhibition of NO by the crude extracts of *G. pentaphylla* have been reported to inhibit NO radicals *in-vitro*[50].

This study also demonstrated that the TPC, and TFC of EEGPle were higher than EEGPst, and EEGPro. Hence, this study recommends that polyphenolic constituents may be the chief agents for the antioxidant action. The variation in the polyphenolic contents may be due to the existence of volatile/essential oils present in the plant. Numerous studies have also suggested that the medicinal plants showed antioxidant activity due to the presence of ploy-phenolic and flavonoid compounds [51, 52].

CONCLUSION: The consumption of synthetic antioxidant rich diet has resulted to cause adverse effects on human beings. The present study suggests that ethanolic leaf extract of *G. pentaphylla* have a potent antioxidant activity. In summary, this study clearly revealed that the leaf extracts of *G. pentaphylla* leaf have a significant antioxidant activity as compared to stem and root extracts and may be beneficial for preventing free radicals mediated oxidative stress. It has also been seen from the crude drug analysis that the plant can be used for the preparation of drugs. As a result, the leaf extracts of *G. pentaphylla* leaf may serve as a possible source of natural antioxidant. Further, detailed phytochemical studies to isolate the compound/s responsible for antioxidant activity has to be done.

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REFERENCES:

1. Dixon GR and Aldous DE: Horticulture: Plants for people and places. 1st ed. New York: Production horticulture Springer 2014.
2. Sacco LN: Drug enforcement in the United States: History, policy, and trends. 1st ed. Washington, DC: Congressional Research Service 2014.
3. Ibrahim EA, Gul W, Gul SW, Stamper BJ, Hadad GM, Salam R and Radwan MM: Determination of acid and neutral cannabinoids in extracts of different strains of *Cannabis sativa* using GC-FID. Plantamedica 2018; 84(4): 250-59.
4. Phillipson JD: Phytochemistry and medicinal plants. Phytochemistry 2001; 56(3): 237-43.
5. Wu JQ, Kosten TR and Zhang XY: Free radicals, antioxidant defence systems, and schizophrenia. ProgNeuro-Psychoph 2013; 46(1): 200-06.
6. Taniyama Y and Griendling KK: Reactive oxygen species in the vasculature: molecular and cellular mechanisms. Hypertension 2003; 42(6): 1075-81.
7. Dhawan BN: Centrally acting agents from Indian plants. In: Koslow SH, Murthy RS, Coelho GV, editors. Decade of the Brain: India/USA Research in Mental Health and Neurosciences. Rockville: National Institute of Mental Health 1995; 197-202.
8. Yoganarasimhan SN and Jadhav D: Medicinal plants of India. 1st ed. Bangalore: Interline Publishers 1996.
9. Chopra GS: Man and marijuana. Int J Addict 1969; 4(2): 215-47.
10. Ahmad I and Aqil F: *In-vitro* efficacy of bioactive extracts of 15 medicinal plants against ESβL-producing multidrug-resistant enteric bacteria. Microbiol Res 2007; 162(3): 264-75.
11. Nayak SA, Kumar S, Satapathy K and Naik SK: *In-vitro* plant regeneration from cotyledonary nodes of *Withania somnifera* (L.) Dunal and assessment of clonal fidelity using RAPD and ISSR markers. Acta Physiol Plant 2013; 35(1): 195-203.

12. Amran H, Farhana R, Shapna S, Rumana M and Apurba SA: Antimicrobial, antioxidant and cytotoxic effects of methanolic extracts of leaves and stems of *Glycosmis pentaphylla* (Retz.) Correa. J App Pharm Sci 2011; 8(1): 137-40.
13. Sarkar R and Mandal N: *In-vitro* cytotoxic effect of hydroalcoholic extracts of medicinal plants on Ehrlich's Ascites Carcinoma (EAC). International Journal of Phytomedicine 2011; 3(3): 370.
14. Gupta N, Agarwal M, Bhatia V, Jha SK and Dinesh J: *In-vitro* antioxidant activity of crude extracts of the plant *Glycosmis pentaphylla* Correa. Int J Pharm Sci Rev Res 2011; 6(1): 158-62.
15. Snyder HL and Kirkland JJ: Introduction to Modern Liquid Chromatography, 1st ed. New York: John Wiley 1979.
16. Sofowora A: Medicinal plants and traditional medicinal in Africa. 3rd ed. Nigeria: John Wiley 1982.
17. Harborne JB: Phytochemical methods: a guide to modern techniques of plant analysis. 2nd ed. New York: Chapman and Hall 1973.
18. Suriyamoorthy P, Subrhamanian H and Kanagasapabathy D: Comparative phytochemical investigation of leaf, stem, flower and seed extracts of *Macrotyloma uniflorum* L. Indo Ame J Pharma Rese 2014; 4(11): 5415-19.
19. Al-Daihan S, Al-Faham M, Al-Shawi N, Almayman R, Brnawi A and Zargar S: Antibacterial activity and phytochemical screening of some medicinal plants commonly used in Saudi Arabia against selected pathogenic microorganisms. J King Saud Uni-Sci 2013; 25: 115-20.
20. Kapoor LD, Singh A, Kapoor SL and Shrivastava SN: Survey of Indian medicinal plants for saponins, alkaloids and flavonoids. LIoydia 1969; 32: 297-302.
21. Smolenski SJ, Silinis H and Farnswoth NR: Alkaloids screening. Lloydia 1974; 37: 506-36.
22. Krishnamoorthi R: Phytochemical screening and antioxidant activity of Justici*a tranquebariensis* and *Bauhinia racemosa*. Int J Pharmacog 2015; 2(7): 362-67.
23. Blois MS: Antioxidant determinations by the use of a stable free radical. Nature 1958; 29: 1199-1200.
24. Tiwari P, Kumar B, Kaur M, Kaur G and Kaur H: Phytochemical screening and extraction: A review. Int Pharmaceutica Sciencia 2011; 1(1): 98-104.
25. Chang CH, Lin HY, Chang CY and Liu YC: Comparisons on the antioxidant properties of fresh, freeze-dried and hot-air-dried tomatoes. J Food Eng 2006; 77(3): 478-85.
26. Fellegrini N, Ke R, Yang M and Rice-Evans C: Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying 2, 2′-azinobis (3-ethylenebenzothiazoline-6-sulfonic acid radical cationdecolorization assay. In Methods in enzymology 1999; 299: 379-89.
27. Garrat DC: The quantitative analysis of drug. 3rd ed. Japan: Chapman and Hall 1963.
28. Cheung LM, Cheung PC and Ooi VEC: Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem 2003; 81: 249-55.
29. Kim DO, Jeong SW and Lee CY: Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem 2003; 81: 321-26.
30. Park YS, Jung ST, Kang SG, Heo BK, Arancibia-Avila P and Toledo F: Antioxidants and proteins in ethylene-treated kiwifruits. Food Chem 2008; 107: 640-48.
31. Yuan H, Ma Q, Ye L and Piao G: The traditional medicine and modern medicine from natural products. Molecules 2016; 21(5): 559.
32. Ames BN, Shigenaga MK and Hagen TM: Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci 1993; 90(17): 7915-22.
33. Vinson JA, Jang J, Dabbagh YA, Serry MM and Cai S: Plant polyphenols exhibit lipoprotein-bound antioxidant activity using an *in-vitro* oxidation model for heart disease. J Agr Food Chem 1995; 43(11): 2798-99.
34. Deb D, Datta B, K, Debbarma J and Deb S: Ethno-medicinal plants used for herbal medication of jaundice by the indigenous community of Tripura, India. Biodiversitas 2016; 17(1): 256-59.
35. Hussein HK, Elnaggar MH and Al-Zahrani NK: Antioxidant role of folic acid against reproductive toxicity of cyhalothrin in male mice. Glo Adv Res J Environ Sci Toxicol 2012; 1(4): 066-071.
36. Dwivedi S and Udupa N: *Terminalia arjuna*: pharmacognosy, phytochemistry, pharmacology and clinical use. A review. Fitoterapia 1989; 60(5): 413-20.
37. Valette G, Sauvaire Y, Baccou JC and Ribes G: Hypocholesterolaemic effect of fenugreek seeds in dogs. Atherosclerosis 1984; 50(1): 105-11.
38. Norberg A, Hoa N, K, Liepinsh E, Van Phan D, Thuan N, D and Jörnvall H: A novel insulin-releasing substance, phanoside, from the plant *Gynostemma pentaphyllum*. J BiolChem 2004; 279(40): 41361-67.
39. Lamkadem M, Melhaoui A, Mimouni J and Fontonge R: Cytotoxic effect and electrophysiologic activity of (R)-bgugaine, an alkylpyrrolidine alkaloid against MRC-5 fibroblasts. Toxicon 2001; 39(4): 485-90.
40. Oyedemi SO, Yakubu MT and Afolayan AJ: Antidiabetic activities of aqueous leaves extract of *Leonotis leonurus* in streptozotocin induced diabetic rats. J Med Plant Res 2011; 5(1): 119-25.
41. Sindhu S and Manorama S: GC-MS determination of bioactive components of *Polycarpaea corymbosa* lams (Caryophyllaceae). Hygeia JD Med 2013; 5(1): 5-9.
42. Gan RY, Xu XR, Song FL, Kuang L and Li HB: Antioxidant activity and total phenolic content of medicinal plants associated with prevention and treatment of cardiovascular and cerebrovascular diseases. J Med Plant Res 2010; 4(22): 2438-44.
43. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L and Byrne DH: Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Compos. Anal 2006; 19(6-7): 669-75.
44. Nadia B and Benmahdi H: Phytochemical Study: Antioxidant activity of *Euphorbia resinifera* L. Adv Tech Biol Med 2013; 1: 104.
45. Račková L, Májeková M, Košt'álová D and Štefek M: Antiradical and antioxidant activities of alkaloids isolated from *Mahonia aquifolium*. Structural aspects. Bioorgan Med Chem 2004; 12(17): 4709-15.
46. Ge L, Lin B, Mo J, Chen Q, Su L and Li Y: Composition and antioxidant and antibacterial activities of essential oils from three yellow Camellia species. Trees 2019; 33(1): 205-12.
47. Luo A and Fan Y: Antioxidant activities of berberine hydrochloride. J Med Plants Res 2011; 5(16): 3702-07.
48. Gupta, DK, Inouhe, M, Rodríguez-Serrano, M, Romero-Puertas, MC and Sandalio LM: Oxidative stress and arsenic toxicity: role of NADPH oxidases. Chemosphere 2013; 90(6): 1987-96.
49. Smith-Palmer, A, Stewart, J and Fyfe L: The potential application of plant essential oils as natural food preservatives in soft cheese. Food Microbiology 2001; 18(4): 463-470.

50. Bhatia, A, Bharti, SK, Tewari, SK, Sidhu OP and Roy R: Metabolic profiling for studying chemotype variations in *Withania somnifera* (L.) Dunal fruits using GC–MS and NMR spectroscopy. Phytochemistry 2013; 93: 105-15.
51. Khorasani-Esmaeili A, Mat Taha R, Mohajer S and Banisalam B: Antioxidant activity and total phenolic and flavonoid content of various solvent extracts from *in-vivo* and *in-vitro* grown *Trifolium pratense* L. (Red Clover). Biomed Res Int 2015; 1: 643-85.
52. Pandey KB and Rizvi, SI: Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev 2009; 2(5): 270-78.

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