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Standardization and comparative evaluation of phytochemical content and antioxidant activity of Alocasia indica and Tephrosia purpurea

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***Abstract*---**Extracts of leaves of *Tephrosia purpurea* and tuber of *Alocasia indica* were used as a traditional medicine in India for the management of various diseases, including rheumatic arthritis, hepatoprotective activity and antifungal properties. The objective of the work is to standardize the phytochemical constituent and comparatively analyze the anti-oxidant activity of *Alocasia indica* and *Tephrosia purpurea.* Leaves ethanolic extract of *Tephrosia purpurea* and tuber ethanolic extract of *Alocasia indicia* Schott. were evaluated for antioxidant activity using 1, -diphenyl-2-picryl hydrazyl (DPPH) assay. Phenolic content was estimated by using Folin-Ciocalteu's

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reagent. Total flavonoid content was measured for *Alocasia indica* and *Tephrosia purpurea* by the aluminum chloride colorimetric assay. Furthermore, Albino mice were used to test the acute toxicity of the plant extracts. The standardization of plant extracts demonstrated lesser amount of oil-soluble contents is present in both the plants. The total flavonoid contents were 76.93±1.41 and 107.44±1.89 mg QE/g while the total phenolic contents were 512.33±1.52 and 595.66±2.51 mg GAE/g for *A. Indica* and *T. Perpurea,* respectively. The antioxidant assay illustrated, significant DPPH radical scavenging potency of plant extracts. No sign of toxicity or mortality was observed at a dose of 1000 mg/kg and 2000 mg/kg for *A. Indica* and *T. Perpurea,* respectively.The results indicated that *Tephrosia purpurea* has significantly higher flavonoid content and so, higher antioxidant effect as compared to *Alocasia indica* thus, it can be used in management of various oxidative stress induced diseases.

***Keywords*---**alocasia indica schott, tephrosia purpurea, antioxidant, standardization, phytochemical evaluation.

Introduction

Medicinal plants are used for the improvement of various disorders since the primeval era as traditional medicine. Medicinal plants are primary sources of bioactive compounds and used for discovery of new drug [1–4]. They play a significant role in development of modern medicine. With the advancement and invention in the area of drug development of human civilization and knowledge, therapeutic uses of the herbals have been increased. Researchers always took an attempt to perform pharmacological tests to identify and isolate the actives which are beneficial for the human begins for treatment of various diseases [5–7].

Alocasia indica Schott belongs to family Araceae, commonly famous as Elephant Ear Taro or Gaint Taro. The plant is a vigorous green herb of height up to 1.8 m and widely distributed in Asia and the Pacific islands [6,8]. The leaves are previously reported as hepatoprotective, digestive, laxative, diuretic, antifungal, astringent, and useful in rheumatic arthritis. The plant has mainly flavonoids, cyanogenetic glycosides, ascorbic acid, gallic acid, malic acid, oxalic acid, alocasin, amino acids, succinic acid and β-lectins [5,9].

Tephrosia purpurea (L.) Pers. belongs to family Fabaceae [10], its common name is Sarapunkha. This plant is vastly branched, sub-erect, herbaceous perennial [11,12]. In Ayurveda, it is known as 'wranvishapaka' means property of healing the entire wounds. It is the most important part of ayurvedic preparations Tephroli and Yakrifit used to treat liver disorders. This plant is also used for asthma, impotence, diarrhea, rheumatism, gonorrhea, ulcers, urinary and heart diseases [13,14]. The dried herb is effective as a tonic laxative, diuretic, and deobstruent. The plant has mainly glycosides, rotenoids, isoflavones, flavanones, chalcones, flavanols, and sterols [15,16].

Material and Methods

DPPH (1, 1diphenyl, 2 picryl hydrazyl) was procured from Sigma Chemicals USA and rest of the chemicals and solvents used are of analytical grade. Folin-Ciocalteu's reagent was purchased from Sigma Chemical Co. Ltd. Ascorbic acid and Gallic acid procured from Loba Chemicals Ltd, Mumbai.

Collection of plant material

The *Alocasia indica* and *Tephrosia purpurea* were collected from the district of Raipur, Chhattisgarh in December 2015. Stay away from any adulteration during collection. Botanical recognition was performed by Professor N. K. Dubey, Department of Botany, Faculty of science, Varanasi (U.P.), where a voucher specimen (Accession No. Ara. 2018/1 and Papiliona 2018/1) has been submitted for further reference.

Extraction

The fresh plant was collected and shaded dried, followed by coarsely powdered to 60-mesh size and processed for extraction. Dried sample (500 g) was two times extracted (2000 ml) 95% ethanol at 45°C for 48 hr and rotary evaporator (IKA RV10) was used to concentrate the solution under reduced pressure at 40°C. Then this sample was placed in amber-colored glass containers.

Standardization of Plant materials

Estimation of foreign matter

Plant-based raw material should be free from any fecal traces attributed to molds, insects or animals and also it does not have any abnormal odor, discoloration, and deterioration. Standard guidelines should be followed to check these above parameters. Exactly 250g of the sample was spread over a plane platform and observed for any unwanted material by a magnifying lens (10x). The remnants are passed through a sieve no. 250 and dust were observed as a mineral admixture. If any material is observed, then it should be weighed in grams and expressed as per 100g of the air-dried sample [17,18].

Determination of ash values

The amount of material remains after ignition is referred to as total ash, which includes both physiological ash (from the plant tissue) and non-physiological ash (from extraneous matter). Acid-insoluble ash could be defined as the resultant matter after boiling the total ash within dilute HCl* and if any insoluble matter remains, then it was subjected to re-ignition [18,19]. It referred as the amount of silica that was observed most often as sand and siliceous earth [19].

* hydrochloric acid

Total ash

Accurately weighed 4g of the sample was put into the previously ignited silica crucible. The sample was spread as a thin layer and ignited by gradually increasing the temperature up to 500-600°C followed by cooling through desiccator and weighed. Total ash fraction was denoted in terms of mg/g of dry material [18,19].

Acid-insoluble ash

25 ml of hydrochloric acid was added into silica crucible that prior contains total ash and after 5 minutes boiling; 5 ml of hot water was added. Then the insoluble matter was collected on ashless filter-paper followed by washing with warm water until neutral filtrate was obtained. The filter paper with insoluble matter was then placed in a new crucible, previously dried on a hot-plate and ignited until it reaches a constant weight. Then by the use of desiccator, the residue was cooled for 30 minutes and then weighed instantly. It was then expressed as mg/g of air-dried material [20].

Water-soluble ash

25 ml water was added into the crucible having total ash, and after 5 minutes boiling, insoluble matter if any was collected on a sintered glass crucible, washed with warm water and ignited in a crucible for 15 minutes below 450°C. The weight of the above residue in mg was deducted from the total ash weight. Then it was expressed as mg/g of air-dried material [20].

Determination of swelling index

Approximately 1 g of finely powdered samples were transferred to measuring cylinder (25 ml), then after adding 25 ml of tap water, this mixture is subjected to vigorous stirring for every 10 minutes to 1hr. Then the resultant mixture was kept aside for 3 hr at room temperature. The quantity in ml was measured which was absorbed by the reduced powder material. The mean value of each determination was calculated [20–22].

Determination of foaming index

1g of finely powdered materials was transferred into a conical flask (500ml) priorly contains 100ml hot water and heated the mixture at 45°C for 30 minutes. The cooled and filtered decoction was placed into a volumetric flask (100ml) and volumes were made up to 100 ml. The decoction was then transferred to 10 stoppered test tubes in successive portions (1-10 ml) and shake for few second and kept aside for 15 minutes and the height of foam is measured [23].

The foaming index was calculated by using the formula = **1000/A**
A= Decoction volume in ml used for making the dilution in the tube where foaming height of 1 cm was observed.

Moisture content

Accurately weighed 5 gm fine ground sample was subjected to IR moisture balance. The loss in weight was noted as percentage (%) moisture with respect to an air-dried sample of the crude drug.

Anti-oxidant Assay

The antioxidant activity of the extract was estimated by DPPH† method. The crude ethanolic extract of the tuber of *Alocasia indica* and leave of *Tephrosia purpurea* is mixed with ethanol for stock solution preparation (100µg/ml). From this stock solution, five sample solutions were prepared to achieve the concentrations of 200 to1000µg/ml. DPPH solutions was poured in every individual test samples and after 20 minutes absorbance was taken at 517 nm. Ascorbic acid was taken as standard drug and the DPPH solution without sample was used as control. The scavenging activity free radical was measured as decrement in absorbance of the samples versus DPPH standard solution. Ethanol was taken as blank [6,16,24,25].

Percent scavenging of the DPPH free radical was calculated using the following formula:

$$\% \text{ DPPH Radical Scavenging Activity} = [1-(As/Ac)] \times 100$$

Ac = absorbance of the control,
As = absorbance of sample solution
The % DPPH Radical scavenging Activity of the extract was plotted against different concentrations [26,27].

Estimation of Total Flavonoid Content (TFC)

TFC of *A. indica* and *T. purpurea* extracts were measured by aluminum chloride colorimetric assay. 2 mL small portion of the extract was mixed with 0.2 mL of sodium nitrite (5%). Then after 5 minutes, 0.2 mL of aluminum chloride (10%) was added to this mixture. After 6 minutes, 2 mL of 1M sodium hydroxide was poured to this mixture. The final volume of the reaction mixture was made up to 5 mL with ethanol (50%). Absorbance at 510 nm against a blank was taken. The TFC was evaluated by a standard curve of quercetin (0-50 mg/mL) and the results were showed as quercetin equivalents [28].

Estimation of Total phenolic Content (TPC)

TPC was evaluated by the newly improved method of Singleton and Rossi and denoted as milligram/gram GAE‡. 100 µL small portions of the sample extracts (1 mg/mL) was then mixed with 2 mL of 20 g/L Na2CO3 solution. After incubation (for 2 mins), 100µ L of Folin-Ciocalteu reagent (50%) was added and the resultant mixture was kept aside for 30 mins at room temperature. The absorbance was taken at 750 nm by a spectrophotometer (UV 1800, Shimadzu Co., Ltd.). The

† 2,2-diphenyl-1 picrylhydrazyl
‡ gallic acid equivalents

blank solution was consisting of all reagents and solvents without the sample. The total phenolic content was calculated by using the standard gallic acid calibration curve. For the gallic acid, the curve absorbance versus concentration is described by the equation

$$y = 0.0013x - 0.0063 \ (R2= 0.9957)$$

where, y = absorbance and x = concentration [29].

Acute toxicity

In albino mice, the acute toxicity of both extracts has been determined. The animals were kept on fasting condition, a night before the test. The animals have been divided into eight groups. For the acute toxicity study [16], the extract was administered orally to different mice groups in increased doses ranging from 1000, 1200, 1400, 1600, 1800, 2000 and 2500 mg/kg.

Results and Discussion

Standardization of Plant materials

The foreign matter, ash and extractive values were determined in percent of plant material of *A. indica* and *T. purpurea* shown in table 1. Acid-insoluble ash measures the quantity of silica present. Water-soluble and alcohol soluble ash represents the approximate amount of soluble phytoconstituents present in the plant material. The lower content of acid insoluble ash between 1.13±0.20 and 2.8±0.11 represents a very minute content of silica and other earth matter. Sulfated ash values represent the presence of metal oxides in small quantities of about 3.01± 0.35% in *A. indica* and 10.83±0.35% in *T. purpurea.* The presence of metals can be attributed to the nature of soil and use of pesticides.

Low acid-insoluble ash value may also affect the absorption when taken orally. The percent extractive values were determined in various solvents ranging of varying polarities. The findings were depicted in terms of percentage extractive values in Table 1. The extractive values of *A. indica* tuber and *T. purpurea* leave was found to be comparatively higher in alcoholic mediums as compared to non-polar solvents. The lower values of chloroform soluble extractive values suggest a lesser amount of oil-soluble contents in the plant [30]. The foaming index, swelling index and moisture content were presented in table 1.

Table 1. Standardization parameters of Plant materials

Parameters		***A. Indica***	***T. Purpurea***
Foreign matter (%w/w± S.D (n=3)		1.13±0.19	1.17±0.13
Moisture content (%w/w± S.D (n=3)		75.2±3.00	9.56±0.32
Extractive Value (% w/w ± S.D) n= 3	Ethanol	3.17± 0.18	4.89± 0.17
Ash content (% w/w ± S.D) n= 3	Total ash content	3.93±0.45	8.74±0.40
	Acid insoluble ash	1.13±0.20	2.8±0.11
	Water-soluble ash	1.76±0.15	1.8±0.25

	Sulfated ash	3.01± 0.35	10.83±0.35
Swelling Index (% w/w ± S.D) n= 3		3.69± 0.67	2.59± 0.53
Foaming Index (% w/w ± S.D) n= 3		0.95± 0.15	3.04± 0.31

Total Flavonoid and Total phenolic Content

Phenolic and flavonoid are the major phyto-group constituents present in the medicinal plant having varying effects like anti-cancer, anti-oxidant, anti-inflammatory, and hepatoprotective. The TP§ and FC** of ethanolic extracts of both the plants were reported in table 2. The ethanol in plants, *A. indica* and *T. Purpurea* exhibited a higher proportion of phenolic and flavonoid contents [31].

Table 2. Determination of total phenolic and flavonoid content of plant sample

Sr. No.	Plant sample	Extract	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
1	*Alocasia Indica* (Tuber)	95% Ethanol	512.33±1.52	76.93±1.41
2.	*Tephrosia Purpurea* (Leave)	95% Ethanol	595.66±2.51	107.44±1.89

Anti-oxidant Assay

DPPH radical is frequently used as an agent in detecting hydrogen-donation capacity and antioxidant activity. Table 3 shows the IC50 values of different extracts as DPPH radical scavenging activities. The findings showed that all the extracts could scavenge radicals of DPPH. Five concentrations between 200 to 1000 µg/ml of the ethanolic extract of *A. indica* and *T. purpurea* have been screened for their capacity for antioxidant. It was noted that at distinct levels free radicals were scavenged by test compounds. The maximum IC†† of *A. indica* and *T. purpurea* found to be 7.05 and 9.60 µg/ml. Several researches have been reported that the importance of phenolic and flavonoid components in oxygen-free radicals scavenging. The extracts showed the presence of flavonoid compound like quercetin, which can be credited for their antioxidant activity [32,33].

Table 3**.** Antioxidant potential of ethanolic extract of *Alocasia indica* and *Tephrosia purpurea*

Sr.No	Plant sample	Extract	Standard Absorbance AA‡‡ (µg/ml)	Different antioxidant models % Scavenging at different concentration (µg/ml)						
				Test extract of sample						
			200	50	100	200	400	800	100	IC5

§ total phenolic
** flavonoid contents
†† inhibitory concentration
‡‡ Ascorbic acid

									0	**0**
1	*Alocasia Indica* (Tuber)	95% ethanol	88.75	38.10	42.47	51.10	59.14	61.31	71.33	7.05
2	*Tephrosia Purpurea* (Leave)	95% ethanol	88.75	32.77	51.29	61.33	69.44	71.36	81.94	9.60

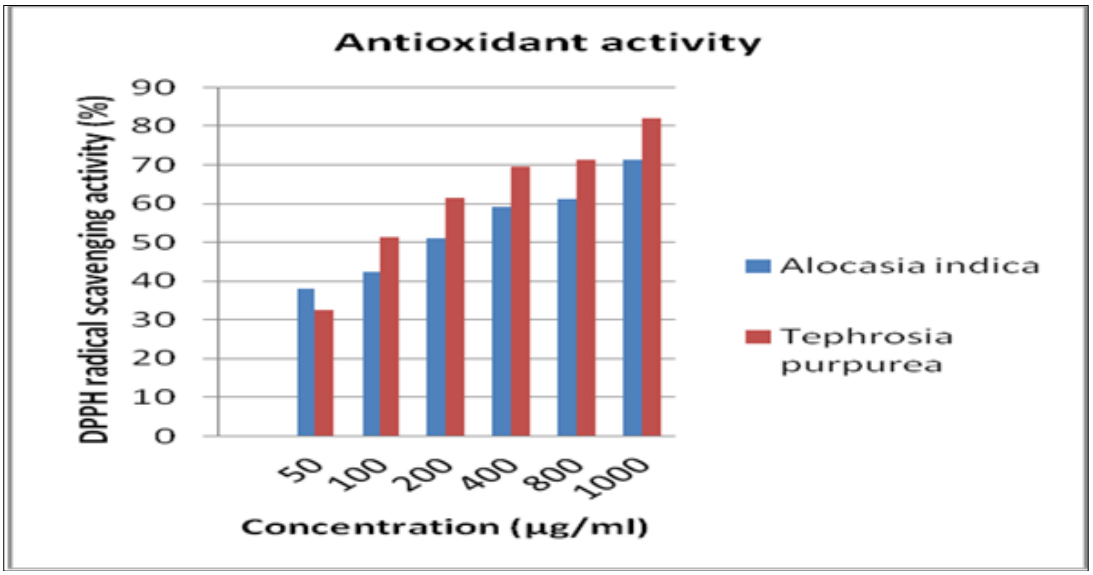

Figure 1: Antioxidant capacity of *A. indica* and *T. purpurea* plants sample

Toxicity Study

Acute toxicity study for each extract was showed no mortality at the dose level 1000 mg/kg body weight for *A. indica* ethanolic extract and 2000 mg/kg body weight for *T. Purpurea* ethanolic extract, after an observation period of 7 days. So, it can be determined minimum lethal dose for extracts is more than 1000 mg/kg and 2000 mg/kg respectively.

Conclusion

The present study indicated that *Tephrosia purpurea* have more significant phenolic and flavonoid content as compared to *Alocasia indica*. Both the plants were standardized with different methods such as ash value, swelling index, foaming index, and investigated for antioxidant activity. Among both the plants, *Tephrosia purpurea* showed the considerable antioxidant effect which might be due to high flavonoid content. In this modern era, most of the diseases are induced and turned in more severe form by oxidative stress and *Tephrosia purpurea* can play an essential role in the management of diseases caused by oxidative stress.

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Conflict of Interest: None

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