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RESEARCH ARTICLE

PHYTOCHEMICAL, TOTAL PHENOLIC CONTENT AND ANTIOXIDANT STUDY OF LEAF AND FRUIT EXTRACTS FROM SELECTED PLANTS WITH DIFFERENT EXTRACTION SOLVENTS

***Haleem Khan, A. A., Naseem, B. and Vidya Vardhini**

Department of Botany, Telangana University, Nizamabad-503322, India

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ABSTRACT

The plant based foods are rich in nutrients and dietary fibers that improve health. The plants are consumed as leaves, flowers and fruits in raw salads and cooked multiple cuisines. The leafy vegetables from amaranth are inexpensive in cost though enriched with phytochemicals that support healthy body. In the present study, extracts from leaves of *Amaranthus gracilis* Desf., *A. tricolor* L., *A. polygamus* L., *Alternanthera repens* L., *A. sessilis* L., *Aerva lanata* L., (*Amaranthaceae),* and from fruits of *Benincasa hispida* (*Thunb.) Cogn.* (Cucurbitaceae), *Capsicum annum* L., *Citrus aurantium* L., were evaluated for organoleptic characters, extractive value, phytochemical constituent, total phenolic content and antioxidant activity. The solvents used for the extraction of plant material were polar and non-polar to check the solvent that yield more phytochemicals. The organoleptic characters of leaf and fruit extracts were found to have acceptable sensory properties. The extractive values (%) showed the suitable solvent applicable for phytochemical extraction. The phytochemical constituents under study were detected in selective solvents due to their solubility. The total phenolic content of leaf and fruit extracts were high with alcoholic solvents, and hence based on the findings from our studies, it can be aptly suggested that alcohols are ideal solvents for extraction of phytochemicals. This study reveals that the extracts are moderate potential sources of natural antioxidants.

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INTRODUCTION

The changes in lifestyle have altered the dietary preferences among the majority of population of all ages. The changes in food consumption have brought common disorders like hyperglycaemia, hypertension and obesity. The reactive oxygen species (ROS) or free radicals are known to cause cellular oxidation that result in various chronic diseases such as diabetes, cardiovascular disorders and cancer. The antioxidants protect from ROS attack that target in biological cells (Ibrahim *et al*., 2015). The evidences of antioxidants to stabilize or deactivate free radicals played a pivotal role for their use in prevention and treatment of diseases and maintenance of human health. The antioxidants either in the form of raw extracts or their chemical components are very effective to avoid the destructive processes caused by oxidative stress (Munir *et al*., 2014). The commonly used synthetic antioxidants viz. butylated hydroxyanisole (BHA), Propyl gallate (PG), butylated hydroquinone and butylated hydroxytoluene (BHT) are available but due to toxic affect that cause DNA damage, use of synthetic antioxidants is restricted (El-kassas *et al*., 2015).

****Corresponding author: Haleem, A. A.***
Department of Botany, Telangana University, Nizamabad-503322, India.

As per the estimate of alternative systems of medicine like Ayurveda, Siddha, Unani and Homeopathy, approximately 70, 000 plant species were proven for medicinal components (Upadhya *et al*., 2015). There are large numbers of medicinal plants reported for their antioxidant properties. In response to stress conditions, plants synthesize secondary metabolites mostly phenolics that improve resistance. These plant phenolics may be soluble or insoluble and complex in nature. The phenolic compounds in fruits, vegetables, spices and herbs are prominent source of antioxidants for humans (Hossain and Shah, 2015). The plants belonging to the genus Amaranthus, consists of about 60-70 species and mostly they grow as annual weeds. These plants were common due to easy in cultivation, fast growth rate; adapt to different agro climates with high yield potential. These are among the common leafy vegetables used in multiple cuisines. *Amaranthus* spp. has been used in treatment of liver infections, knee pain, laxative, and diuretic properties. The products of *Amaranthus* spp. are reported for their use in stomach aches, diarrhoea, and dysentery (Aphalo *et al*., 2015). *Benincasa hispida* (Thunb.) Cogn. (ash guard) fruit was reported to contain triterpenoids, flavonoids, phenolic compounds, tannins, proteins, amino acids, carbohydrates, glycosides, saccharides, carotenes, vitamins, β-sitosterol and uronic acid. The fruits were reported to treat nervous disorders, GIT problems (dyspepsia and burning sensation), heart

disease, vermifuge, diabetes and urinary disease (Nadhiya *et al*., 2014). The genus Capsicum comprises more than 200 varieties, native to the tropical and humid zone. The fruits vary widely in size, shape, flavor and sensory heat and are used as a spices, food, and therapeutic applications like treatment of arthritis, rheumatism, stomach ache, skin rashes, dog bite, snake bite and wounds. Capsicum *spp.* are known to contain carotenoids, capsaicinoids (lipophilic alkaloid with analgesic and anti-inflammatory activity), and phenolic compounds (flavonoids, quercetin and luteolin) (Chen and Kang, 2013). The genus *Citrus* is known as source of edible fruits such as oranges, mandarins, limes, lemons and sour oranges. These fruits are rich source of bioactive compounds such as phenolics (e.g., flavanone glycosides, hydroxycinnamic acids), vitamin C, and carotenoids. The fruit extracts were reported for inhibitory activities (anti-inflammatory, antitumor, antifungal, and blood clot inhibition) (Wang *et al*., 2014). In the present study extracts from leaves of *Amaranthus gracilis* Desf., *A. tricolor* L., *A. polygamus* L., *Alternanthera repens* L., *A. sessilis* L., and *Aerva lanata* L., (*Amaranthaceae),* fruits of *Benincasa hispida* (*Thunb.) Cogn.*, *Capsicum annum* L., *Citrus aurantium* L., were evaluated for organoleptic characters, extractive value, phytochemical constituents, total phenolic content and antioxidant activity.

MATERIALS AND METHODS

Plant materials

Leaves: *Amaranthus gracilis* Desf., *A. tricolor* L., *A. polygamus* L., *Alternanthera repens* L., *A. sessilis* L., and *Aerva lanata* L. (*Amaranthaceae),* Fruits: *Benincasa hispida* (*Thunb.) Cogn.* (Cucurbitaceae), *Capsicum annum* L. (Solanaceae), *Citrus aurantium* L. (Rutaceae). The plant materials were purchased from local vegetable market. The plants were identified and authenticated with the help of Flora (Gamble, 1935).

Chemicals and equipment

All the chemicals and reagents used in the study were analytical grade procured from SD Fine Chem. Ltd., Qualigens, and Hi-media, India. The equipment used was purchased from Systronics and Remi, India.

***Extraction*:** The samples (leaves and fruit pulp) were separated, then dried separately at 50°C, pounded to coarse powder and filtered through sieve mesh. 100 g coarse powder was used to prepare extracts with ethanol, ethyl acetate, methanol, sterile distilled water.

***Organoleptic characters*:** Various sensory parameters of the plant material were evaluated.

***Extractive value*:** 4g coarse powder was placed in a glass-stoppered conical flask and macerated with 100 ml of the solvent for 6 h, shaking frequently, and then allowed to stand for 18 h. The mixture was filtered and 25ml of the filtrate was transferred to a tared flat-bottomed dish and evaporated to dryness on a water bath. The residue was dried at 105°C for 6 h, cooled in a desiccator for 30 min, and weighed.

Extractive value = Initial weight-Final weight/Initial weight ×100

Phytochemical screening

Table 1. Methods for phytochemical screening

S. No	Phytochemical	Method
1	Alkaloids	Mayer's
2	Amino acids	Ninhydrin
3	Flavonoids	Ferric chloride
4	Glycosides	Keller-Kiliani
5	Saponins	Foam
6	Tannins	Gelatin

Estimation of total phenolic content (Singleton and Rossi, 1965): 20 µl of sample extracts (ethanol, ethyl acetate, methanol, sterile distilled water) (5 mg/ ml) was mixed thoroughly with 0.75 ml of 20% sodium carbonate solution and 0.25 ml of Folin-Ciocalteau reagent. The reaction mixture was allowed to stand in light for 3 min and incubated for 1 h in dark at room temperature. The absorbance was measured at 760 nm using UV-visible spectrophotometer. The concentration of total phenolics was quantified by calibration curve obtained from measuring the absorbance of known concentrations of gallic acid standard. The concentrations were expressed as µg of gallic acid equivalents (GAE) per ml. The extracts were analyzed in triplicates and values were averaged.

Determination of DPPH radical scavenging activity (Burits and Bucar, 2000): Two ml of freshly prepared methanol solution of 1, 1- diphenyl-2-picrylhydrazyl (DPPH) (0.004%) was added to 20 µL of extracts and allowed to stand at room temperature for 30 min. The absorbance (A) of sample solution was measured at 517 nm, compared with that of control solution (maximum absorbance). Control solution was prepared containing the same volume without any extract. Ascorbic acid was used as standard (positive control). The extracts were analyzed in triplicates and values were averaged. Scavenging percentage of the DPPH free radical was measured using the following equation: DPPH radical scavenging (%) = $[(A_C – A_S)/ A_C] \times 100$

A_C: absorbance control, A_S: absorbance sample

RESULTS AND DISCUSSION

The plants under study are sold in vegetable market after initial rain. The women from villages around the urban locality collect these plants that are weeds. These plants grow fast in the farm lands and very much available in months of Aug-Oct. The green leaves are used as vegetarian cuisine, people suffering from liver disorders like jaundice prefer to consume. The local herb healers crush fresh green leaves administer orally in cow or goat milk to patients. Hence this study was designed to evaluate the phytochemicals that contribute the healing property of these plants. The organoleptic characters are sensory parameters like taste, color, and odor were studied. The amaranth leaves were bitter in taste and green in color (Table 2), *B. hispida* fruit was palatable in taste and odor was pleasant, *C. annuum* fruit was hot in taste with pungent odor, *C. aurantium* was pleasant in odor with sweet taste (Table 3). The extractive value (%) for leaf sample from *A. gracilis* was 18-32 and it was high in methanolic extract. The leaf sample (%) extractive value for *A. tricolor* (17-32), *A. polygamous* (19-31), *Alternanthera repens* (17-35), *Alt. sessilis* (15.2-36) and *Aerva lanata* (17-34). These sample extractive value (%) were high in ethanolic extract (Table 2). In *B. hispida* fruit peel (18-32), pulp (17.2-32), seeds (19-31), *C. annuum* fruit peel (18-32), pulp (19-31), seeds (19-30), *C. aurantium* fruit peel (18-32), pulp (19-31), seeds (19-30), ethanol was better solvent to yield high extractive value (Table 3).

Table 2. Extractive values [%] of Amaranth leaves

Solvents	*A. gracilis*	*A. tricolor*	*A. polygamous*	*A. repens*	*A. sessilis*	*A. lanata*
Ethanol	30	32	31	35	36	34
Ethyl acetate	28	29	24	3 0	29	27
Methanol	32	24	29	30	28	28
Distill water	18	17	19	17	15.2	17

Table 3. Extractive values [%] of Fruits

Solvents		*B. hispida*	*C. annuum*	*C. aurantium*
Ethanol	Peel	30	32	32
Ethyl acetate		28	28	28
Methanol		32	31	31
Distill water		18	18	18
Ethanol	Pulp	32	31	31
Ethyl acetate		29	29	29
Methanol		24	29	29
Distill water		17.2	19	19
Ethanol	Seeds	31	30	30
Ethyl acetate		24	27	27
Methanol		29	25	25
Distill water		19	19	19

Table 4. Free radical scavenging effect of Amaranth leaf extracts by DPPH method

Solvents	*A. gracilis*	*A. tricolor*	*A. polygamous*	*A. repens*	*A. sessilis*	*A. lanata*
Ethanol	74 ± 0.15	83± 0.21	89± 0.51	80± 0.48	90± 1.05	90± 0.37
Ethyl acetate	64± 0.16	72± 0.06	67± 0.31	88± 0.30	86± 0.14	87± 0.24
Methanol	73± 0.10	83± 0.02	76± 0.07	81± 0.05	89± 0.41	78± 0.17
Distill water	51± 0.05	61± 0.01	56± 0.09	65± 0.01	52± 0.06	55± 0.12

Table 5. Free radical scavenging effect of Fruit extracts by DPPH method

Solvents	*B. hispda*	*C. annum*	*C. aurantium*
Ethanol	85± 0.35	95± 0.44	85± 0.44
Ethyl acetate	78± 0.17	80± 1.05	89± 0.41
Methanol	85± 0.35	85± 0.35	78± 0.17
Distill water	62± 0.16	64± 0.09	51± 0.01

The results of phytochemicals showed presence of alkaloids, amino acids, flavonoids, glycosides, saponins, tannins in leaf extracts of *A. gracilis*, *A. tricolor, A. polygamus* leaf extracts showed presence of three phytochemicals, *Alt. sessilis* extracts showed four phytochemicals and extracts of *Aerva lanata, Alternanthera repens* showed five phytochemicals. The phytochemicals such as alkaloids, amino acids, flavonoids, glycosides, saponins, tannins present in fruit extracts of *B. hispida*. *C. annuum* and *C. aurantium* showed three phytochemicals. The total phenolic content in leaf extracts (ethanol, ethyl acetate, methanol, sterile distilled water) were found to be 68-85, 66-80 and 56-85 µg/ml for *A. gracilis, A. tricolor* and *A. polygamus* (Figure 1). The leaf extracts of *Alternanthera repens, Alt. sessilis* and *Aerva lanata* total phenolic content was 15-45, 14-40 and 20-40 µg/ml (Figure 1). The fruit pulp extracts of *B. hispida*, *C. annuum* and *C. aurantium* was estimated to be 56-85, 66-80 and 68-85 µg/ml (Figure 2). The total phenolic content was high in *A. gracilis* and *A. polygamous* leaf extracts and in *C. aurantium* fruit pulp extracts. The plant material under study for total phenolic content was high in alcoholic extracts and low with distilled water. The ethanolic leaf extracts of *A. sessilis, Aerva lanata* and fruit pulp extracts of *C. annuum* were high in free radical scavenging ability. The alcoholic extracts showed high free radical scavenging ability than aqueous extracts for the plant material under study (Table 4, 5). The seeds and sprouts of *Amaranthus cruentus* were reported for total antioxidant capacity, phenolic contents and anthocyanins. The increase in growth period of sprouts increased the antioxidant capacity, 4 days growth period showed high antioxidant activity, (Pasko *et al*., 2009). The methanolic seed and sprout extracts of amaranth were reported for polyphenols and *in vitro* antioxidant activity. The seed and sprout extracts improved the bread making process. The amaranth sprouts were alternative source that enhance the nutritive property of gluten-free bread (Jubete *et al*., 2010). The methanolic and aqueous seed extracts of *A. cruentus* showed different antioxidant capacity in thermal and enzymatic treatments of methanolic and aqueous seed extracts. The antioxidant capacity was high with enzymatic treatment and was better than thermal (Pazinatto *et al*., 2013). The leaf extracts of red amaranth (*A. tricolor*) and green amaranth (*A. viridis*) were evaluated for *in vitro* antioxidant potential at different temperatures and pH. The increase in temperature and pH yielded high antioxidant capacity with both leaf extracts (Pramanik *et al*., 2014). The leaf extracts of red amaranth (*A. tricolor*) cultivars were evaluated for total phenolic content and antioxidant activity by growing the plants at different sunlight level. The full sunlight was found better antioxidant activity than plants grown in shaded conditions. The specific growth conditions were found to improve the antioxidant properties in leaf extracts (Khandaker *et al*., 2008, Ratnakar *et al*., 2013). The aqueous stem extracts of *Aerva lanata* (L.) Juss. ex Schult were reported to contain phytochemicals like carbohydrates, oils, fats, saponins, flavonoids, alkaloids, tannins and phytosterols. The extracts were found with high amount of total phenolic content (Kumar *et al*., 2013, Sharma *et al*., 2010, Rajesh *et al*., 2011). The methanolic and aqueous extracts from aerial parts of *A. lanata*

were reported for antioxidative and antimicrobial activities. These extracts were rich in saponins, flavonoids and tannins. The inhibition of oxygen derived free radicals, reducing power, nitric oxide scavenging activity was observed with methanolic extracts and the activities were concentration dependent (Muthukumaran *et al*., 2011, Raihan *et al*., 2012). The ethanolic and ethyl acetate leaf extracts of *A. lanata* were studied for antioxidant, inhibition of alpha glucosidase, protein glycation dipeptidyl peptidase IV, protein tyrosine phosphatase, stimulation of glucose uptake and glitazone (adipogenic) potential (Riya *et al*., 2015, Raghavendran *et al*., 2012).

industries (Abdullah *et al*., 2012). Benamrouche et al. reported the phenolic contents and antioxidant activity with the peels and leaves of orange varieties (*Citrus sinensis* L. and *C. aurantium* L.). The extracts were found as source of anti-free radical activity (Benamrouche *et al*., 2013, Sarrou *et al*., 2013). The methanolic peel extracts of Tunisian *C. aurantium* L were reported for bioactive compounds, antioxidant activity and heat stability (Karoui *et al*., 2014). The oil from seeds of bitter orange (*C. aurantium* L.) and mandarin (*C. reticulata* Blanco) were collected at the different stages of seed maturity. The extracts were evaluated for lipid composition and antioxidant capacity.

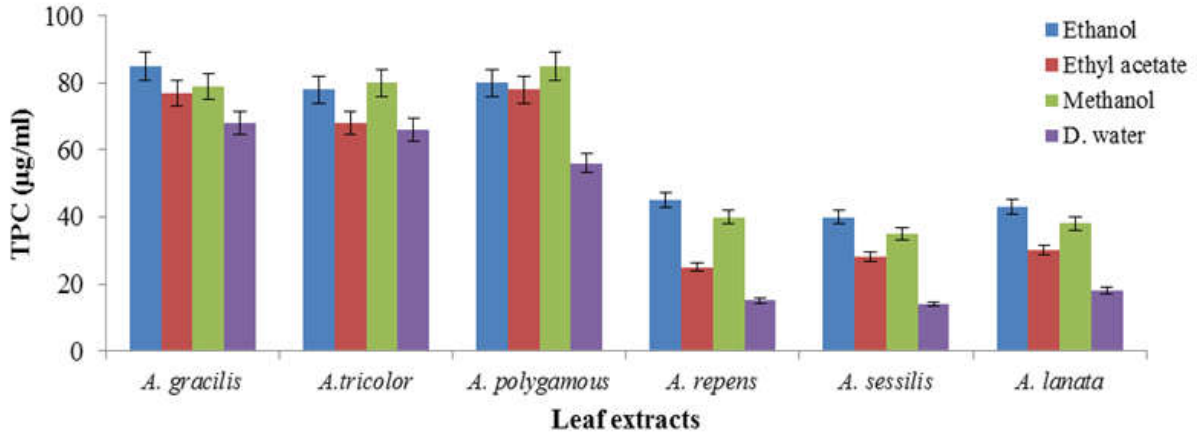

Figure 1. Total phenol content (TPC) of Leaf extracts

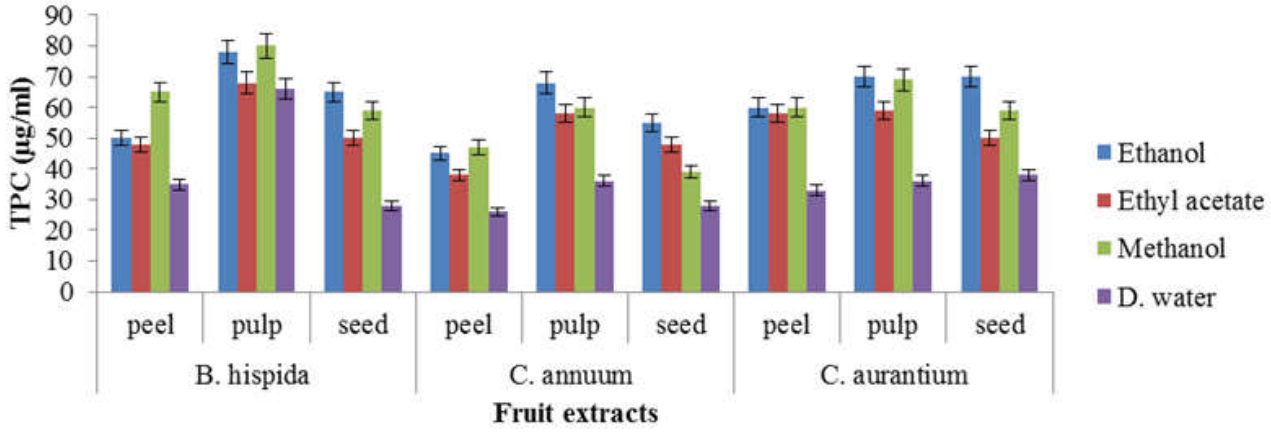

Figure 2. Total phenol content (TPC) of Fruit extracts

The aqueous leaf extracts of *Alternanthera sessilis* (Linn.) were evaluated for alkaloids, ascorbic acid, carbohydrates, proteins, tannins, anti-microbial and antioxidant activities. The reducing power for silver nanoparticle synthesis was ascorbic acid (Niraimathi *et al*., 2013). The stem and leaf extracts of *A. sessilis* with methanol, acetone, and ethanol were reported for *in vitro* antioxidant and free radical scavenging activity. The methanolic extracts showed increased antioxidant activity in concentration dependent manner (Borah *et al*,. 2011, Hossain *et al*., 2014, Ogundare *et al*., 2014).

The pulp, peel and seed extracts of *Benincasa hispida* (Thunb.) were evaluated for antioxidant and antimicrobial activities. The seed extracts were strong in antioxidant capacity and antibacterial activity. The extracts were proved as potential natural preservatives for food, cosmetic and pharmaceutical

The compounds such as ascorbic acid and butylated hydroxyl toluene (BHT) were compared for the antioxidant capacity with seed oil of bitter orange and mandarin. The antioxidant activities were harvesting time-dependent (Tounsi *et al*., 2011). The essential oil from *C. aurantium* L. and monoterpene limonene (flavoring agent) were evaluated for their effect on gastric mucosa. The essential oil and flavor agent were found gastroprotective (Moraes *et al*., 2009). The hot water fruit peel extracts of *C. poonensis* Hort. Ex Tanaka and *C. unshiu* Marc were reported for presence of flavonoids, narirutin, nobiletin, tangeretin, hesperidin, flavone glycosides, polymethoxylated flavones, phenolic acids and minerals (K, Mg, Ca, Cu, Fe, Mn, Zn). The extraction of minerals and phenolic compounds was found better at temperature-100°C for 30 min (Xu *et al*., 2008, Rekha *et al*., 2010). The peel and fruit juice of Tunisian *C. aurantium* were reported for bioactive contents and antioxidant

capacity (Karoui and Marzouk, 2013, Barreca *et al*., 2011). The methanolic extracts of 4 different colored (red, orange, yellow, green) bell peppers (*Capsicum annuum* L) were reported for antioxidant activity and anti-proliferative action. The study showed red and orange peppers were high in total phenolic content and the orange pepper variety was high in free radical scavenging activity. The superoxide dismutase (SOD) activity was high in green pepper (Park *et al*., 2012). The volatile oils extracted from *Capsicum annuum* L. were evaluated for chemical composition and antioxidant capacity. These oils extracted from pepper fruit was proved to be novel natural antioxidant and flavoring agent (El-Ghorab *et al*., 2013, Wahua *et al*., 2014). The carotenoids were extracted from fruits of *Capsicum annuum* L. and were evaluated for antioxidant, analgesic and anti-inflammatory activities. The analgesic and anti-inflammatory activities of carotenoids proved to be useful for treatment of pain and inflammation (Ortega *et al*., 2012, Perucka and Materska, 2007).

Conclusions

The plant based foods are rich in nutrients and dietary fibers that improve health. The plants are consumed as leaves, flowers and fruits in raw salads and cooked multiple cuisines. The leafy vegetables from amaranth are inexpensive in cost though enriched with phytochemicals that support healthy body. The solvents used for the extraction of plant material were polar and non-polar to check the solvent that yield more phytochemicals. The organoleptic characters of leaf and fruit extracts were found to have acceptable sensory properties. The extractive values (%) showed the suitable solvent applicable for phytochemical extraction. The phytochemical constituents under study were detected in selective solvents due to their solubility. The total phenol content of leaf and fruit extracts were high with alcoholic solvents, hence based on the findings from our studies alcohols are solvents ideal for extraction of phytochemicals. This study reveals the extracts are moderate potential source of natural antioxidants.

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