Evaluation of antioxidant activity of leaves and fruits extracts of five medicinal plants

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Abstract: Antioxidants acts as a defense mechanism that protects against oxidative damage caused by free radicals produced in the body. Medicinal plants are preferably used for various diseases in many countries. The studies were conducted to determine the antioxidant capacity of the ethanolic leaves and fruits extracts of *Physalis minima, Withania somnifera, Datura inoxia, Solanum nigrum* and *Kigelia africana* by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. Quercetin was used as a standard antioxidant which shows 93.66% inhibition. Among the five selected plant species, the percentage of antioxidant activity of leaves extracts was found in order: *P. minima* > *W. somnifera* > *S. nigrum* > *K. africana* > *D. inoxia* and fruits extracts was in order: *W. somnifera* ≥ *D. inoxia* > *P. minima* > *K. africana* > *S. nigrum* respectively.

Keywords: Antioxidant activity, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), quercetin, ethanolic leaves and fruit extracts.

INTRODUCTION

Antioxidants prevent the oxidation caused by free radicals and sufficient intake of antioxidants is supposed to protect against diseases (Celiktar *et al.,* 2007). The body produces many antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidise, which neutralize many types of free radicals (Gamiotea-Turro *et al.,* 2004). Antioxidants can act by scavenging reactive oxygen species (superoxide dismutase removing oxygen), by inhibiting their formation (e.g. by blocking activation of phagocytes) and preventing formation of OH and/or decomposition of lipid hydroperoxides, by repairing damage or by any combination of the above (Niwa *et al.,* 2001). Oxidative stress is a factor for many human diseases, as either a cause or an effect. Plants are the source of medication for preventive, curative, protective or promotive purposes (Sidhu *et al.,* 2007). The best health and nutrition results can be achieved not only from the consumption of fruits and vegetables with high antioxidant capacities, but also from medicinal herbs and plants (Jastrzebski, 2007).

Family Solanaceae comprises of approximately 84genera and 3000species, such as potato, petunia, nightshade and tobacco (Zygadlo *et al.,* 1994). It is a cosmopolitan family found throughout tropical and temperate regions of the world. *S. nigrum* is a low branched annual herb, having triangular stems, alternate leaves and tiny white flowers. Fruit is green in color when immature and turns to purplish black when ripe (Kothekar, 1987). *P. minima* is also an annual herb which yields high fruit (Patel *et al.,* 2011). It has small, round fruit and comprises of 150-300 seeds usually enclosed in bladder- like calyx (Peter, 2007). *W. somnifera,* commonly known as Indian ginseng and winter cherry, is an evergreen, erect, branching shrub having simple leaves and greenish or lurid yellow flowers and fruits are orange red when mature and are globose berries (Anonymous, 2007). *D. inoxia,* commonly known as devil's turnip and dhatora, also belongs to family Solanaceae (Stevens *et al*., 2001).

Kigelia africana is the member of family Bignoniaceae. It is also known as 'bamkheera' in Hindi. It is found in wetter areas and spread across wet savannah and riverine area (Cragg and Newman, 2001). The tree is up to 20 m tall and bark is smooth and grey in colour while it starts peeling on older trees (Roodot, 1992). The leaves are 30-50cm long, and opposite or in whorls of three. The fruit is a woody berry that is 30-100cm long and 18cm broad; weight can be 5-10 kg hangs down on a long rope like peduncles (Joffe, 2003). It is fast growing and can mature in 4 to 5 years. It begins to flower from the age of 6 years. Mature fruits can be found on trees throughout the year (Jackson and Katie, 2012). Extracts of *Kigelia* plant have potent anti-oxidant activity due to caffeic acid and its derivatives (Olaleye and Rocha, 2008).

MATERIAL AND METHOD

Apparatus and chemicals

The apparatus used for antioxidant activity include ELISA (Enzyme-linked immunosorbent assay), electric balance (Sartorius-GE 412), rotary evaporator (Butchi-Switzerland Rotavapor-R210), 96-well plate, cuvettes, micropipette (Thermo scientific-Finnpipette F3), petri dishes, conical flasks, beakers (Atlas Borosilicate-Glass 3.3), filter paper (Whatman filter paper-1), nylon bags and

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mortar and pestle. The chemicals used were ethanol, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and quercetin.

Preparation of samples

Leaves and fruits were carefully washed with tap water and rinsed with distilled water. All plant materials were spread in a clean stainless tray and air-dried under shade at room temperature for 10-15days. Dried leaves were crushed and ground into coarse powder with mortar and pestle and the shade-dried fruits were powdered in the electric grinder. The powdered material was kept in nylon bags.

Preparation of extracts

The simple extraction procedure was adopted for these medicinal plants. 10g each of dry powdered plant materials were soaked in 100mL of ethanol at room temperature for 36 hours. Here, the extraction was done with solvent under shaking conditions. The respected extracts were then filtered through a Whatmann filter paper, in order to obtain an aqueous extract and solvent was removed completely under reduced pressure. Filtered extract was collected in a conical flask. Then the ethanol extract was evaporated by rotary evaporator at 45ºC to get the crude extracts for the determination of antioxidant activity (Sigaroodi *et al.*, 2008).

Evaluation of antioxidant activity

The percentage of antioxidant activity (AA%) of each extracts was assessed by DPPH free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams *et al.* (1995). The samples were reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted of adding 5µL of sample, 95 µL of DPPH radical solution 0.5mM in ethanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read absorbance at 517nm after 100min of reaction using ELISA. The mixture of ethanol and sample serve as blank. The control solution was prepared by mixing ethanol and DPPH radical solution. The scavenging activity percentage (AA%) was determined according to Mensor *et al.* (2001).

$$AA\% = 100 - \left[\frac{(Abs_{sample} - Abs_{blank})x100}{Abs_{control}}\right]$$

(Abssample is the absorbance of the sample, Absblank is the absorbance of the blank and Abscontrol is the absorbance of the control). Quercetin was used as a standard antioxidant.

STATISTICAL ANALYSIS

Values were mean ±SD (standard deviation) of three replicates. All experiments were performed at least, three times (unless indicated otherwise) and were highly reproducible. Data collected was analyzed statistically by using statistical software and means were separated by least significant different test at $P<0.05$ (Steel *et al.*, 1996).

RESULTS

The percentage of antioxidant activity of different parts of the respective plants was assessed by DPPH free radical assay. Quercetin was used as a standard antioxidant which shows 94% inhibition. The present studies revealed that the leaves extracts of *P. minima* (76.66%) showed best radical scavenging activity as compared to leaves extracts of other plants. *S. nigrum* showed moderate (62.0%) antioxidant activity while *D. inoxia* exhibited lowest (50.33%) antioxidant activity among leaves extracts of five selected species.

Table 1: Results of antioxidant activity of selected medicinal plants

Plant name	Plant parts	DPPH% inhibition
Physalis minima	Leaves	76.66
	Fruits	70.33
Solanum nigrum	Leaves	62.00
	Fruits	37.66
Withania somnifera	Leaves	69.66
	Fruits	77.33
Datura inoxia	Leaves	50.33
	Fruits	77.33
Kigelia africana	Leaves	59.66
	Fruit	62.66
Quercetin (standard antioxidant)		93.66

Moreover, in case of fruits extracts *W. somnifera* and *D. inoxia* possess good radical scavenging activity (i.e., 77.33 %), whereas lowest (37.66%) antioxidant activity has been observed in the fruits of *S. nigrum* respectively (table 1). However, fruits of *P. minima* possess moderate (70.33%) activity against DPPH respectively. Overall, our results revealed that all the selected medicinal plant species exhibits remarkable antioxidant activity in varying concentrations when their different parts were tested in ethanolic extracts, so all these species can be used as a valuable drug against various antioxidants produced in body (fig. 1).

DISCUSSION

Oxygen is an element obligatory for life where living systems have evolved to survive in the presence of molecular oxygen, which has double-edged properties, being essential for life; it can also aggravate the damage within the cell by oxidative events (Shinde *et al.*, 2012). Oxidative stress results when the balance between the productions of reactive oxygen species (ROS) exceeds the antioxidant capability of the target cell (Ahmad *et al.*,

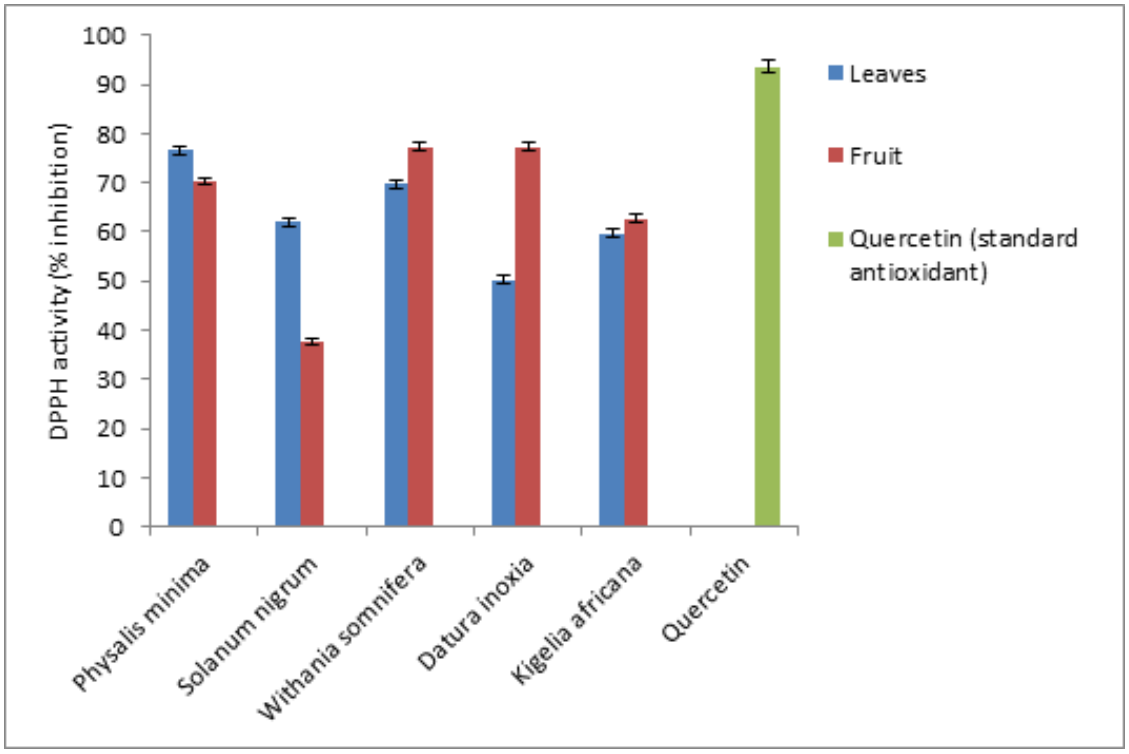

Fig. 1: Antioxidant activity of selected medicinal plant extracts

2009). The plant extracts and their confined constituents have always been an important part of different curative systems (Vanitha and Kathiravan, 2006).

Plants are rich sources for natural antioxidants, the best known are flavonoids, vitamin C and other phenolic compounds (Laandrault *et al.,* 2001). Polyphenols scavenge free radicals and inhibit the oxidative mechanisms that can lead to degenerative diseases. Plants are considered as one of the most important and interesting subjects that should be explored for the discovery and development of newer and safer drug candidates (Hamid *et al.,* 2011). Present studies revealed that leaves extracts of *P. minima* and *W. somnifera* possess significant antioxidant activity as compared to the leaves extract of *K. africana* and *D. inoxia*. While *S. nigrum* exhibit moderate activity. In case of fruit extracts of selected species, *W. somnifera*, *D. inoxia* and *P. minima* showed remarkable activity as compared to *K. africana* and *S. nigrum* respectively. Overall, all these species can be utilized as a drug in pharmaceutical industries. Therefore, the development of alternative antioxidants mainly from natural sources has attracted considerable attention.

CONCLUSION

The antioxidant activity of different plants was screened by DPPH free radical assay by using quercetin as a standard antioxidant which shows 94% inhibition. Overall, the studies revealed that all the selected species possess remarkable DPPH radical scavenging activity in varying concentrations. Hence, these plants have great potential to be developed as drug by pharmaceutical industries. It is suggested that the respective plants could be used as an additive in the food industry providing good protection against oxidative damage. However, further studies are required to find out the exact active components which are responsible for antioxidant activities.

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