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EVALUATION OF *IN VITRO* ANTI OXIDANT ACTIVITY OF DRIED ROOTS OF *ABRUS PRECATORIUS*

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ABSTRACT

Abrus Precatorius is one of the important herb commonly known as Indian licorice belonging to family Fabaceae. The roots, seeds and leaves are used in traditional & folklore Medicine. It is reported to have a broad range of therapeutic effects, like anti-bacterial, anti-fungal, anti-tumor, analgesic, anti-inflammatory, anti-spasmodic, anti-diabetic, anti-serotonergic, anti-migraine, including treatment of inflammation, ulcers, wounds, throat scratches and sores. It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products but still additional information needs to be updated. The plant *Abrus Precatorius* was investigated for their anti oxidant activity. The extractions were subjected to assay by Reducing power, Nitric oxide scavenging activity and DPPH methods for evaluation of anti oxidant activity. The results primarily suggest the presence of potent oxidant inhibitory principles in the roots of *Abrus Precatorius*.

KEYWORDS: Reducing power, anti oxidant, nitric oxide scavenging activity, free radical scavenger & DPPH method.

INTRODUCTION

From the earliest times, herbs have been prized for their pain-relieving and healing abilities and today developing countries still rely largely on the curative properties of plants. According to World Health Organization, 80% of the people living in rural areas depend on medicinal herbs as primary healthcare system. *Abrus precatorius* L. belonging to family Fabaceae is a leguminous climber popularly known as Rati in Hindi, Crab's eye in English,

Gunja in Sanskrit. The plant has been used in Hindu medicines from very early times, as well as in China and other ancient cultures. The seeds have been used to treat fever, malaria, headache, dropsy and to expel worms. A decoction of the seeds is applied for abdominal complaints, conjunctivitis, trachoma and malarial fever. Central Africans use powdered seed as an oral contraceptive. It is also used to lower high blood pressure and relieve severe headache. The seed has purgative properties and is used as anemetic, tonic, aphrodisiac and for nervous disorders.

Abrus –saponins I and II, abrisapogenol, β-amyrin, squalene, abricin, abridine, cycloartenol, campesterol, cholesterol and sitosterol have all been found in the seeds. Proteins – abrins I, II and III, *Abrus precatorius* agglutinin (APA) I and (APA) II are present in the seeds. Alkaloids and nitrogen compounds – precatorine, trigonelline, choline and abrine are present in the seeds. Flavonoids and anthocyanins – abrectorine, dimethoxycentaureidin-7-o-rutinoside, precatorins I, II and III and xyloglucosyl-delophinidin and p-coumaroylgalloyl-glucosyl-delphinidin have been isolated from the seeds.

The seeds possess various pharmacological activities such as anti diabetic, anti oxidative, anti viral, antihelminthic, anti depression, memory enhancing, anti microbial, anti-inflammatory, anti arthritic, anti cancer, anti fertility, anti malarial, anti allergic, anti asthamatic, anti cataract, anti insecticide, anti toxicity activity, Plants synthesize a wide variety of chemical compounds, which can be classified by their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites.

Among the 120 active compounds isolated from the higher plants widely used in modern medicine, 80 percent showed a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived. The Indian Ayurvedic system treasures a host of medicinal plants that have been shown to possess a wide variety of pharmacological activities.

There has been a worldwide positive move towards the use of traditional medicines due to the concern over the more invasive, expensive and potentially toxic main stream modern practices. Its popularity is due to desire more for more personalized health care and greater public access to health information. Anti oxidants may an important role in the prevention of these diseases. There is an increasing interest in the antioxidant effects of compounds derived from plants, which could relevant in relation to their nutritional incidence and their role in

health and disease. A number of reports on the isolation and testing of plant derived antioxidants have been described during the past. We studied different extracts of leaves of *Abrus Precatorius* were investigated for their antioxidant activity by reducing power assay, Nitric oxide scavenging assay, DPPH radical scavenging assay.

Anti oxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions. Free radicals or ROS formed in the body as a result of biological oxidation. Over production of free radicals contributes to oxidative stress associated with various degenerative diseases. Oxidation is very important for life to survive but harmful effects of oxygen are believed to be due to the formation of reactive oxygen species (ROS) known as free radicals, these free radicals have been implicated in the pathogenesis of various diseases including myocardial and cerebral ischemia, arteriosclerosis, diabetes, cataracts, atherosclerosis, rheumatoid arthritis, inflammation and cancer-initiation as well as in the aging process result in tissue damage and cell death.

Botanical description

A woody twinning plant of the leguminosae family with characteristic red and black seeds. The leaves are pinnate and glabrous, with many leaflets (12 or more) arranged in pairs. The leaflets are oblong, measuring 2.5-cm long and 1.5-cm wide. The plant bears orange-pink flowers, which occur as clusters in short racemes that are sometimes yellowish or reddish purple in color, small and typically pealike. The plant produces short and stout brownish pods, which curl back on opening to reveal pendulous red and black seeds, 4 to 6 peas in a pod.

Origin and distribution

It grows wild in thickets, farms and secondary clearings, and sometimes in hedges. It is most common in rather dry areas at low elevation throughout the tropics and subtropics. The plant *Abrus precatorius* Linn popularly known as Rosary pea, jequirity bean belong to the family leguminosae (Fabaceae) is found throughout India in hedges and bushes in exposed areas. The seeds are deadly poisonous but it has been reported that the toxic form of abrin gets converted to mitogenic form upon long refrigerated storage. Usually seeds are of two types one is scarlet with black spot and the other variety is pure white.

PLANT PROFILE

Taxonomical classification

Kingdom : Plantae

Division : Magnoliophyta

Order : Fabales

Family : Fabaceae

Subfamily : Faboideae

Tribe : Abreae

Genus : *Abrus*

Species : *Abrus precatorius*

MATERIALS AND METHODS

Plant Material

The roots of *Abrus precatorius* were collected from surrounding places of Rangareddy Dist. The seeds collected were washed under running tap water, blotted dry and kept for drying in oven at temperature 40 ± 2^0C for five days. The dried seeds were powdered and stored in air tight container. The plant specimens for the proposed study were collected from ken to select healthy plants and for normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Farmalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hrs the specimens were dehydrated with graded series of tertiary butyl alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual additional of paraffin wax (melting point 58-60^{0}C) until tertiary butyl alcohol solution attained super saturation. The specimens were cast into paraffin blocks.

Extraction

10 grams of each plant fine powder of indigenous plants weighed into a 250 ml conical flask and 100 ml of solvents were added separately for each plant powder then on a rotary shaker at 190 – 220 rpm for 24 hours. This was filtered with whatman no. 1 filter paper, the residue discarded and the filter were evaporated to dryness in a water bath temperature at 80^{0}C.

Preparation of stock solution

Stock solution was prepared by weighing 10 mg of each dried solvent extract dissolved in 1 ml of dimethyl sulphoxide (DMSO) giving a final concentration of 10,000 µg/ml. The stock solution was kept in screw capped bottles for further analysis.

Anti oxidant activity

The total anti oxidant activity of the extract was evaluated by the DPPH radical scavenging assay, Nitric oxide radical scavenging assay and Reducing power assay.

DPPH RADICAL SCAVENGING ASSAY

Procedure

DPPH radical scavenging activity was measured by the spectrophotometric method. A stock solution of 25mg of DPPH (150µM) was prepared in 100ml of ethanol, 0.1ml of extract of different concentrations was dissolved in DMSO and 1.9ml of DPPH was added. 0.1ml of DMSO was added to 1.9ml of DPPH in the case of control and 0.1ml of DMSO was added to 1.9ml of ethanol in the case of blank. The reaction was allowed to be completed in the dark for about 20 minutes. Then the absorbance of test mixtures was read at 517nm. The percentage inhibition was calculated and expressed as percent scavenging of DPPH radical. Curcumin (50, 100, 200µg) was used as standard.

NITRIC OXIDE RADICAL SCAVENGING ASSAY

Procedure

The nitric oxide radical scavenging activity of *Abrus Precatorius* was determined according to this method. Aqueous solution of sodium nitroprusside spontaneously generates nitric oxide (NO) at physiological pH, which interacts with oxygen to produce nitrate ions and which was measured colorimetrically. 3ml of reaction mixture containing 2ml sodium nitroprusside, 10mM in phosphate buffered saline (PBS) and 1ml various concentrations of the extracts were incubated at 37^0C for 4 hours. Control without test compound was kept in an identical manner. After incubation 0.5mL of Griess reagent was added. The absorbance of the chromophore formed was read at 546nm. The percentage inhibition of nitric oxide generation was measured by comparing the absorbance values of control and those of test compounds. Curcumin (50, 100, 200µg) was used as standard.

REDUCING POWER ASSAY

Procedure

The reducing power assay of *Abrus Precatorius* was determined according to this method. Extracts of different concentrations were prepared in 1ml of DMSO and mixed with 2.5ml of phosphate buffer (pH 6.6, 0.2M) and potassium ferricyanide (2.5ml, 10%). The mixture was incubated at 50^0C for 20 minutes. Aliquots of trichloroacetic acid (TCA) (2.5ml, 10%) were added to the mixture, which was then centrifuged at 1500 rpm for 10 minutes. The upper

layer of reaction mixture was mixed with distilled water (2.5ml) and freshly prepared $FeCl_3$ solution (0.5ml, 0.1%). The absorbance was measured at 640nm. Increase in absorbance of the reaction mixture indicates the increase in reducing power. Reducing power is given in terms of ascorbic acid equivalent (as Emg^{-1}).

RESULTS AND DISCUSSION

The extracts of *Abrus Precatorius* were assayed for anti oxidant activity for petroleum ether extract and ethanolic extracts equivalent with ascorbic acid and results are presented in figure-1 and figure-2 respectively. The anti oxidants act either by scavenging various types of free radicals derived from oxidative processes, by preventing free radical formation through reduction precursors or by chelating agents. In this study the extracts significantly posses anti oxidant activity.

Table-1: Determination of reducing power and total anti-oxidant activity by *in vitro* method

Extracts (2000 µg)	Reducing power (mg)	Total anti-oxidant power (mg)
Petroleum ether	0.09	6.13
Chloroform	0.3	3.03
Ethyl acetate	0.07	5.26
Alcohol	0.6	4.88
Water	0.2	3.96

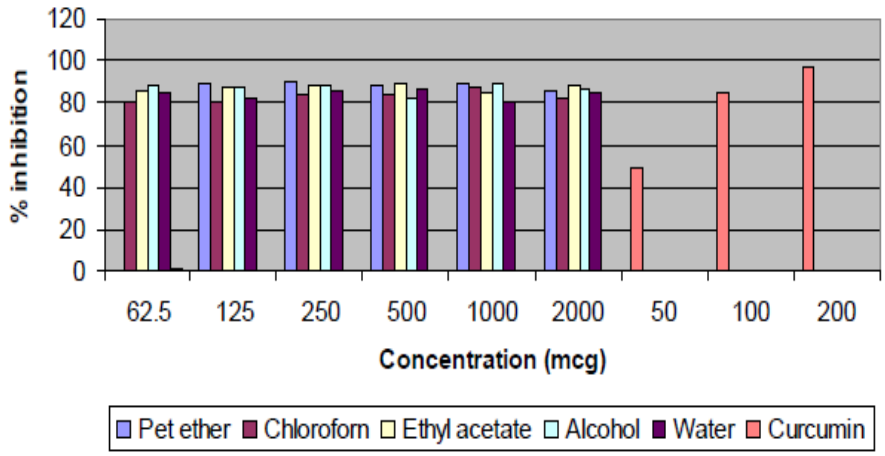

Figure-1: Effect of *Abrus Precatorius* on DPPH radical scavenging activity

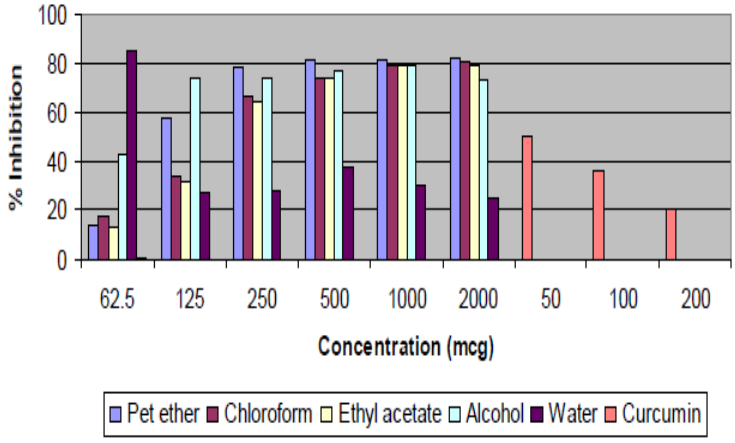

Figure-2: Effect of *Abrus Precatorius* on Nitric oxide radical scavenging activity

CONCLUSION

In conclusion, petroleum ether extract of *Abrus Precatorius* showed maximum anti oxidant property. These revelations are significantly noticeable as numerous metabolic disorders and functional defects might be attributed to its anti oxidant potential. However, scientific confirmation of traditional claims is necessary for exploiting the therapeutic benefits of this wonder herb. In the light of the results of present investigation, it can be concluded that the antioxidant potentials of petroleum ether extract of *Abrus Precatorius in vitro* is promising, however the plant extracts should be investigated to find out the various phytochemicals responsible for anti oxidant and pharmacological activities. However, further studies are required to assess the molecular mechanism of anti oxidant activity of the *Abrus precatorius* L. roots. Furthermore, a detailed and systematic approach can be done in exploiting and identifying the phytopharmacology to explore in knowing the maximum potentiality of the plant which will be useful to mankind. A detailed and systematic study is required for identification, cataloguing and documentation of plants, which may provide a meaningful way for the promotion of the traditional knowledge of the herbal medicinal plants. In view of the nature of the plant, more research work can be done on humans so that a drug with multifarious effects will be available in the future market.

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