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Research Article

EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY, WOUND HEALING ACTIVITY AND ESTIMATION OF PHENOLIC, FLAVONOID AND PROANTHOCYANIDINE CONTENTS OF THE PLANT "*CRATEVA MAGNA*"

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ABSTRACT

Antioxidants are widely reported to prevent occurrence of many inflammatory and metabolic disorders. Pharmacological evaluation of Crateva magna has revealed its potent activity against many diseases. This study evaluates the antioxidant capacity and wound healing activity of three extracts (petroleum ether, chloroform, methanol) were assessed against in vitro free radical scavenging activity by ABTS method, DPPH method and total ferric reductive potential and in vivo wound healing potential in rats respectively, suggesting its role as bioactive enhancer. The present study indicates though all three extract have antioxidant and wound healing activity, but the methanolic extract showed greater and pet ether extract showed the minimum free radical scavenging and wound healing activity. The methanolic extracts of the plant Crataeva magna posses higher phenolic, flavonoid and proanthocyanidine content than the other chloroform and pet ether extract. .

Keywords: *Crateva magna,* Antioxidant activity, Free radical scavenging activity, Ic50

INTRODUCTION

All organisms contain anti free-radical defense system, which includes antioxidant enzymes like catalase, peroxidase and superoxide dismutase and antioxidant like ascorbic acid and tocopherol. The responses of body immune system requires free radicals but it have been shown to be harmful as they react with important cellular components such as proteins, DNA and cell membrane linked to certain chronic diseases of liver, heart and some form of cancers[1,2]. There are more evidences suggesting the antioxidant activity of plants might be due to their phenolic compounds, flavonoid, proanthocyanidine[3]. The process wound healing involves a series of cellular and cytokine mediated events results in the generation of Reactive Oxygen Species (ROS) which have been found to play deleterious role[4]. The surface application of substances with free radical scavenging properties has shown to significantly improve wound healing and protect tissues from oxidative damage[5].

Crataeva magna Buch Ham (family Capparidaceae) is known as three leaved caper in English, Varuna in Sanskrit and Baruna in Hindi, a small tree with a much branched head, found to be distributed mainly in the warmer (tropical) parts of the world. This plant is known to possess immense pharmacological activity-nephrotoxicity[5], arthritis[6], urinary disorders[7]. In folk medicine, its stem pith in the tribal peoples of Kandhamal district of Orissa known as Eastern Ghats of India that the bark is used for lactation after child birth, treat urinary disorders, kidney bladder stones, fever, vomiting and gastric irritation[8,9]. There is lack of enough investigation correlating antioxidant potential to in vivo wound healing activity. Therefore, in the present investigation, we have attempted and correlate, determination of in vitro free radical scavenging property in general to phenolic compounds and in vivo wound healing activity of the plant *Crataeva magna*.

MATERIALS AND METHODS

The leaves of *Crateva magna* were collected in November 2009 in the eastern part of Orissa. The plants were identified by their vernacular names and latter a voucher specimen was validated and deposited at the Department of Botany, Utkal University. The leaves were air dried at room temperature to constant weights. The dried plant materials were subjected for successive extraction process with increasing polarity of solvents i.e. petroleum ether, chloroform and methanol. The extracts were filtered using a Buckner funnel and Whatman no1 filter paper. Each filtrate was concentrated to dryness under reduced pressure at 40˚c using a rotary evaporator.

Chemicals And Glassware

The following reagents were used for the analysis: Butylated hydroxytoluene (BHT), 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picryl-hydrazyl (DPPH) was purchased from HI Media. Ferrous chloride, trichloroacetiacid (TCA), gallic acid, rutin, aluminum trichloride, Folin-Ciocalteu reagent, vanilline were from E. Merck. All chemicals used including the solvents, were of analytical grade.

Determination Of Total Phenolic Content

Total phenolic contents in the extracts were determined by the modified Folin-Ciocalteu method[10]. An aliquot extracts of were mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The tubes were vertexed for 15 sec and allow to stand for 30min at 40° C color development. Absorbance was measured at 765 nm using the Jasco UV-VS spectrophotometer. Samples of extract were evaluated at a final concentration of 0.1mg/ml. Total phenolic content were expressed as mg/g gallic acid equivalent using the following equation based on the calibration curve: $y = 0.12x + 0.203$, $R^2 = 0.30$, where x was the absorbance and y was the gallic acid equivalent (mg/g).

Determination Of Total Flavonoid Content

Total flavonoid were estimated using the method of Ordon ez al[11]. To 0.5ml of sample, 0.5ml of 2% $AlCl_3$ ethanol solution was added. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presences of flavonoids. Total flavonoid content were calculated as rutin equivalent (mg/g) using the regression equation of the calibration curve: $y = 0.51x + 0.099$, $R^2 = 0.411$

Determination Of Total Proanthocyanidin Content

Determination of the total proanthocyanidin was based on the procedure by sun et al[12]. A volume of 0.5 ml of 0.1 mg/ml extract solution was mixed with 3 ml of 4% vanillin-methanol solution and 1.5ml hydrochloric acid; the mixture was allowed to stand for 15 min. The absorbance was measured at 500nm. Total proanthocyanidin content was expressed as rutin equivalents (mg/g) using the equation of the calibration curve: $y = 0.2.699x + 0.030$, $R^2 = 0.957$

ABTS Radical Scavenging Assay

The effect of extracts on ABTS radical was estimated using the method of Re et al.[13]. The stock solution contained equal volume of 7

mM ABTS salt and 2.4 mM potassium persulfate was allowed to stand in dark for 16 hour at room temperature. The resultant ABTS solution was diluted methanol until absorbance of about 0.70 ± 0.01 at 734 nm was reached. Varying concentrations of the plant extracts (1 ml) was reacted with 1ml of the ABTS solution and the absorbance was taken at 734 nm between 3-7 min using the spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of BHT and rutin and the percentage of inhibition calculated as (table2):

Table no-2:effect of extrcts on excision wound contraction in rats

% Closure of excision wound area after days	GROUP			
	Control	Pet ether extract	Chloroform extract	Methanolic extract
4TH	21.33±1.88	22.8±2.197	36.48±1.52	52.24±1.39
8TH	45.28±0.98	58.85±1.01	66.46±0.78	81.34±1.09
12TH	67.66±1.15	69.48±1.27	90.46±1.8	94.69±1.22
16TH	76.03±0.94	76.13±0.69	92.29±0.92	99.14±0.73

ABTS radical scavenging activity (%) = [(Abs $_{control}$ – Abs $_{sample}$) / (Abs $_{control}$)] × 100

Where Abs $_{control}$ is the absorbance of ABTS radical+methanol; Abs $_{sample}$ is the absorbance of ABTS radical+sample extract/standard.

DPPH Radical Scavenging Activity

The free radical scavenging activity of Crateva magna was estimated using the method of Liyana-Pathirana and Shahidi[14]. A solution of DPPH (0.135 mM) in methanol was prepared and 1ml of this solution was mixed with 1ml of varying concentration of the plant extracts. The reaction mixture was vortex thoroughly and left in the dark at room temperature for 30 min. the absorbance of the mixture was measured at 517nm using BHT and rutin as references. The ability to scavenge DPPH radical was calculated as (Fig 2):

(%) of DPPH scavenging activity = [(Abs $_{control}$ – Abs $_{sample}$) / (Abs $_{control}$)] × 100

Where Abs $_{control}$ is the absorbance of DPPH radical+methanol; Abs $_{sample}$ is the absorbance of DPPH radical+sample extract/standard.

Determination Of Ferric Reducing Power

The ferric reducing potential of the extract was assayed as described by Duh et al.[15]. The different concentrations of extracts and the standards, BHT and rutin (0.02-0.10 mg ml^{-1}, 1 ml) were mixed with phosphate buffer (2.5 ml, 0.2 M, ph 6.6) potassium ferricyanide [K_3Fe $(CN)_6$] (2.5ml, 1% w/v). The mixture was incubated at 50º C for 20 min.2.5 ml of TCA (10% w/v) was mixed with 2.5ml distilled water and 0.5ml 0.1% w/v $FeCl_3$. The absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power (Fig 3).

Wound Healing Activity (Excision Wound Model[16])

Albino rats of Wistar strain of either sex weighing 180-220 gm were used for the experiment. Animals were randomly divided into five different groups each of six animals. The back of the animal was shaved and washed with spirit. A circular area of 500 Sq.mm was marked out with Indian ink of the intra-scapular region and its full thickness excised with scalpel and scissor under ether anaesthesia. The first group served as control, which was given the vehicle topically. Second, third, and fourth groups were given methanol extracts combinations topically. Animals were treated with drugs daily, from zero to 20th post wounding day. The wound contractions were measured as total percentage reduction in wound area on alternate days. The progressive decrease in the wound area was monitored periodically by tracing the wound margin on a tracing paper and the area was assessed using graph paper.

Statistical analysis: The experimental results were expressed as mean±standard (SD) of three replicates.

RESULTS AND DISCUSSION

The widest spread secondary metabolite polyphenols in plant kingdom have received much attention as potential natural antioxidant in term of their ability to act as both radical scavenger and metal chelator. The polyphenols are high levels antioxidants[17] due to their redox properties, hydrogen donors, quench active oxygen species and decompose superoxide and peroxides radicals[13]. Result obtained in the present investigation reveals that the levels of all these polyphenols (phenolics, flavonoids and proanthoyanidins) in the methanolic extract among all extracts were significantly higher ($p<0.001$) depicts in the table no-1.

Table no-1: Total phenolic content, flavonoid content, and proanthocyanidine content of different extract of the leaves of the plant crateva magna.

Extract	Total phenolic content(mg of GAE/g)	Total Flavonoid content(mg of quercetin/g)	Total Proanthocyanidine content(mg of rutin/g)
Methanolic extract	4.527 mg/g	7.5242 mg/g	5.063 mg/g
Chloroform extract	1.5697 mg/g	3.1321 mg/g	1.164 mg/g
Petroleum ether extract	2.9183 mg/g	4.8061 mg/g	0.796 mg/g

The result of the evaluation of the antioxidant potential of different extracts of Crateva magna and synthetic antioxidants BHT and rutin through free radical scavenging of ABTS, DPPH and total ferric reductive potential is stated as follows:

The ABTS, a proton radical scavenging is an important attribute of antioxidant that decreases the absorbance maxima at 734nm. The reaction of $ABTS^+$ with free radical scavengers present in the test sample occur rapidly and is assessed by following the decrease in sample absorbance at 734 nm. A concentration dependant activity was observed in this experiment (Fig. 1). The methanolic extract of the Crateva magna were fast and effective scavengers of the ABTS radical than the chloroform and pet ether extract. At a concentration 0.5 mg/ml, the methanolic extract possessed a comparable activity to BHT (92.5 and 97.1%, respectively) where as rutin quenched all the radical at a concentration of 0.05 mg/ml.

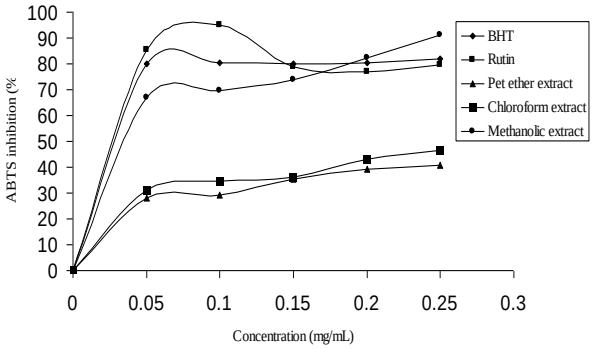

Fig. 1: ABTS RADICAL SCAVENGING ACTIVITY OF EXTRACT AND STANDARD

The DPPH assay is based on the inhibition of the absorbance of the DPPH radical cation. The DPPH scavenging ability of chloroform and pet ether extract is very low as compared to methanolic extract. A significant increase in inhibition of DPPH radical due to the scavenging ability of the methanolic extract and standard BHT and rutin solution (Fig. 2). The DPPH inhibition of the methanolic extract,

BHT and rutin followed in the order, BHT > Rutin > methanolic extract.

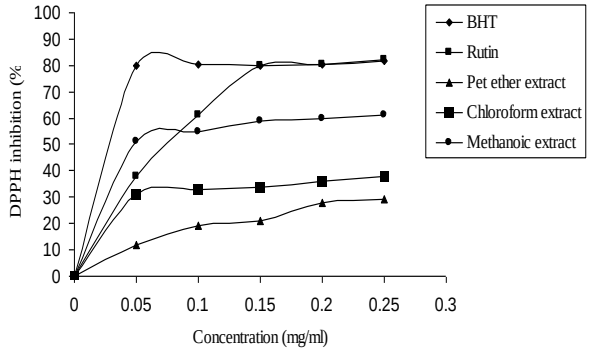

Fig. 2: DPPH RADICAL SCAVENGING ACTIVITY OF EXTRACT AND STANDARD

The reducing power of the extracts, BHT and rutin increased with increasing concentration (Fig. 3) in the following order, BHT > rutin > methanolic extract > chloroform extract > pet ether extract. The presence of antioxidant substance in the extract has been shown to the development of reductions which are terminators of the free radical chain reactors and causes reductions Fe^{3+} to Fe^{2+} form[18].

There was no mortality observed in the course of study. The wound contraction and epithelialisation were faster in methanolic extract followed by chloroform extract followed by pet ether extract when compared to control. In the first two days after wounding, fluid was oozing from the untreated wounds (control) and to some extent, from pet ether treated wounds. But in the other groups the drugs prevented the discharges from the wound. In the drug treated rats the wounds were completely healed in less than 16 days where as in control animals it took more than 26 days. Even on the 8th day the wound contraction was 81% in the treated rats (methanolic extract) where it was only 45% in the control. Statistically analysis showed very encouraging results. When compared with control and drug showed highly significant activity ($p<0.001$). A highly significant activity of Methanolic extract was observed when compared to Chloroform extract ($p<0.01$) and Pet ether extract ($P<0.001$) separately. The bioactivity and better collagenation seen under the influence of these plant extracts may be because of the presence of polyphenols, which is responsible for free radical scavenging activity and is believed to one of the important components of wound healing[19].

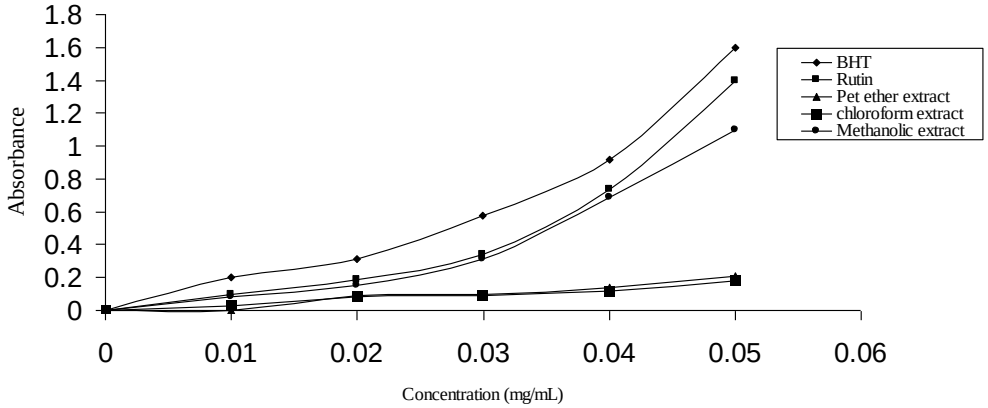

Fig. 3: TOTAL FERRIC REDUCTIVE POTENTIAL OF EXTRACT AND STANDARD

CONCLUSION

The plants *Crataeva magna* may be considered as good sources of natural antioxidants for medicinal uses such as wound healing and other diseases related to free radical mechanisms.

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