



RESEARCH ARTICLE

Protective effect of *Acampe praemorsa* (Roxb.) Blatt. & McCann against oxidative stress

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ABSTRACT

The current study was carried to make available phytochemical information and evaluation of antioxidant activity of *Acampe praemorsa* (Roxb.) Blatt. & McCann. The phytochemical analysis was carried out using procedures and quantified phenolic and alkaloid contents. The antioxidant activity was evaluated by *in-vitro* and *in-vivo* studies. The *In-vitro* antioxidant activity was carried on free radicals such as superoxide, hydroxyl DPPH, hydrogen peroxide, evaluation of reducing power. *In-vivo* study was carried on albino Wistar rats with different doses of extracts. The results provide that, *A. praemorsa* extracts have diversified phytochemicals in extracts like steroids, alkaloids, phenolics, glycosides, oils, quinones, tannins etc. The hydroalcoholic extract has more phenolic (26.80 ± 0.51) and alkaloid (20.59 ± 0.22) contents. The antioxidant activity results provide information that the extracts possess concentration dependent activity on tested free radicals. The hydroalcoholic extract has more protective nature against superoxide, DPPH, H_2O_2 free radicals and reducing power but ethyl acetate extract has more potential against hydroxyl free radical than hydroalcoholic extract. The extracts were found to be safe on toxic studies and *In-vivo* study results and they play significant role in controlling the oxidative enzymes such as catalase, superoxide dismutase, lipid peroxidation (malonaldehyde) in the body. Thus, it was determined that *A. praemorsa* have potential bioactive compounds and antioxidant activity.

Introduction

Now a days, there has been an upsurge of new disease and diverse side effects due to enduring usage of current drugs increasing the interest on evaluation of new therapeutic agents (1). The diseases are mainly affecting functions of different body organs and finally cause mortality (2). The impairment of body organs can lead to imbalance in their actual mechanisms including production of antioxidants (3, 4). The different previous reports explain that maximum diseases source for the over production of oxidants in the body (5, 6). The over production of oxidants can lead to oxidative stress, encompass in progression and development of different diseases like diabetes mellitus, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, arthritis etc (7). The balance between oxidant and antioxidant can increase by providing the external antioxidant as supplements and providing the medications to control oxidants and disease-causing agents in the body (8, 9).

As earlier said, identification of new drugs from different natural resources got more attentive

research around the world and it is primarily from medicinal plants (10). As, the emerging of new diseases and getting resistance to current day using medicines, the researchers and pharmaceutical industries are concentrating on new drugs from plants and their derivatives (11). In recent decades, many bioactive molecules have been reported from medicinal plants around the world to treat different diseases including oxidative stress (12, 13). The discovered medicinal plants have also been used as food supplements to enhance the antioxidants in the body (14). But still, there were many medicinal plants available and not reported scientifically about their medicinal values. *Acampe praemorsa* (Roxb.) Blatt. & McCann (The small warty acampe) is one of such medicinal plants belonging to the family Orchidaceae. Very few researches reported on its traditional usage in bone fractures, anti-typhoid and some other reports about the orchids have potential medicinal values like enhancing white blood cells, reducing headache, fatigue etc. (15-19). So, the current study was aimed to provide the phytochemical information and antioxidant activity of *A. praemorsa*.

Materials and Methods

Chemicals and Reagents

The chemicals and reagents used in current study were analytical grade. The diagnostic kits used in study were purchased from Span Diagnostics Ltd, Gujarat, India. The standard drugs Ascorbic acid, Trolox were from Sigma Aldrich Co.

Preparation of Plant Extracts

The plant material *A. praemorsa* (Roxb.) Blatt. & McCann (Voucher specimen number:23342) was collected from Araku valley region, Visakhapatnam and was authenticated by Prof. S. B. Padal, Department of Botany, Andhra Pradesh, India (Fig. 1). The collected aerial parts were cleaned under running tap water to remove debris and was shade dried. The dried material was made as bristly powder. The powder was used for extraction by maceration process successively using ethyl acetate and hydro-alcoholic solution (70% ethanol in water v/v). The collected solvents were then evaporated using rotavap and extracts [*A. praemorsa* ethyl acetate extract (APEAE) and *A. praemorsa* hydro-alcoholic extract (APHAE)] were stored in desiccator for further usage.

Phytochemical Analysis

The collected extracts were analysed to explore extracts' chemical profile using standard phytochemical tests qualitatively (20, 21) and quantitatively quantified the alkaloids and phenolics.



Fig. 1. *Acampe praemorsa*

Quantification of Phenolic and Alkaloid Contents

Phenolic Content Analysis

The phenolic content was analysed using Folin-Ciocalteu reagent (FCR) as described (22). The method is colorimetric method, based on chemical reduction of reagent mixture containing tungsten and molybdenum. To the extract (mg/ml) added FCR (5 ml) and after the 30 min incubation time, color (blue) of the reaction mixture measured at 760 nm, presence of phenolic content will enhance the absorbance and was calculated against the standard graph of gallic acid and expressed as gallic acid equivalents (mg/g) (21). The results were showed as mean±SEM (n=3).

Alkaloid Content Analysis

The alkaloid contents of selected plant extracts were quantified by spectroscopic method (23) using bromocresol green (BCG) solution. The procedure was, to 1 ml plant extracts dissolved in 2N hydrochloric acid (mg/ml) added 5 ml BCG solution in a separation funnel and 5 ml of phosphate buffer and mixed well. After, the complex formed was extracted (separated) in chloroform (5 ml). The absorbance of chloroform (yellow color) was measured at 470 nm against the standard graph atropine (21). The results were showed as mean±SEM (n=3).

In-vitro Antioxidant Activity

The *in-vitro* antioxidant activity of selected plant extracts was evaluated on superoxide, hydroxy, 2,2-diphenyl-1-picrylhydrazyl (DPPH) (21), hydrogen peroxide (H₂O₂) (24) free radicals and reducing antioxidant power using ferric ion (25). The extracts were dissolved in dimethyl sulphoxide (DMSO) for easy solubility. The experiments results were presented as mean±SEM. The percentage inhibition (PI) was calculated as $PI = \frac{(A_0 - A_1)}{A_0} \times 100$ A₀: Absorbance of control; A₁: Absorbance of plant extract or/and Ascorbic acid. The 50% inhibition of concentrations (IC₅₀ values) were calculated as graph plotted with concentration on X-axis and percentage inhibition on Y-axis.

Superoxide Free Radical Scavenging Activity

Superoxide free radical scavenging activity was assessed as per the method (26). This is a spectroscopic method, evaluating the absorbance of light at 560 nm of a solution containing generated superoxide free radicals' riboflavin with nitroblue tetrazolium (NBT) reduction of color with different concentrations of extracts (20-320 µg/100 µl). The ascorbic acid was used as positive control and the values was measured against corresponding blank.

Hydroxyl Free Radical Scavenging Activity

Hydroxyl scavenging activity of selected plant extracts was carried out by the procedure described (27). The method involved, measuring the absorbance of thiobarbituric acid reactive substances at 532 nm from the reduction of generated hydroxyl radicals through Fenton reaction mixture (Fe²⁺/EDTA/H₂O₂ system).

DPPH Free Radical Scavenging Activity

The DPPH free radical scavenging activity was measured using the standard procedure (28). The procedure was measuring absorbance of alcoholic DPPH (0.004%) (blue color to yellow color) after the addition of 0.1 ml of testing extracts/ascorbic acid at different concentrations (20-320 µg/100 µl).

Hydrogen peroxide Scavenging Activity

H₂O₂ scavenging activity was measured using method described (29). The method is measuring absorbance of reaction mixture at 230 nm containing 0.1 ml of plant extract, 0.3 ml of 50 mM phosphate buffer and 0.6 ml of 2 mM H₂O₂ (24) against the blank.

Reducing Antioxidant Power Assay

The reducing antioxidant power of extracts was measured with spectroscopic method described using Trolox as standard drug (25). The method is measuring color complex absorbance at 593 nm for reduction power of extracts at different concentrations from Fe³⁺ (color less) to Fe²⁺ (blue color) against the blank.

In-vivo Antioxidant Activity

The *In-vivo* antioxidant activity was studied using albino Wistar rats of either sex weighing from 200-250 gm around 60-90 day aged. During the course of study, animals were maintained under controlled conditions (12 hr light/dark cycle, 24±2 °C, 40-70% relative humidity) by supplying sufficient food and water. Prior to start antioxidant activity on animals, the extracts were tested for their toxicity as per Organization for Economic Co-Operation and Development (OECD) guidelines with 1000 and 2000 mg/kg body weight orally on overnight fasting animals using four groups (n=6 each group) and observed at regular intervals for any changes in animals such as skin, morbidity, aggressiveness, oral secretions, sensitivity, pain, respiratory problems and finally mortality. The animal studies were approved by institutional ethical committee of Santhiram Medical College and General Hospital (897/PO/RE/S/05/CPCSEA).

After toxicity study, animals were divided in to seven groups (n=6). Group I served as control received distilled water (0.5 ml), groups II to IV received ethyl acetate extract (100-400 µg/ml) and groups V to VII received hydroalcoholic extract (100-400 µg/ml) of *A. praemorsa* for 21 days administered orally using metal oropharyngeal cannula. 24 hrs after last dosage, blood was collected from animals using direct cardiac puncture under isoflurane anaesthetic condition to get serum through centrifuge at 2500 rpm for 15 min. to measure lipid peroxidation, estimation of superoxide dismutase (SOD) and catalase activity (30, 31).

Lipid Peroxidation

Lipid peroxidation was determined as per the procedure by measuring the thiobarbutiric acid reactive substances (TBARS) and malonaldehyde (MDA) in serum (32). The procedure was, serum was deproteinized by addition of trichloroacetic acid and thiobarbutiric acid and added 0.1 ml of testing

extract and then mixture was heated for 30 min. in water bath. After cooling the mixture was centrifuged at 2000 rpm for 10 min, the absorbance of the supernatant (TBARS) at 535 nm spectrophotometrically against blank. The concentration of TBARS was calculated using the molar extinction coefficient of malondialdehyde (1.56 × 10⁵ mol/L/cm) and results were expressed in nmol/mg of protein.

Superoxide Dismutase (SOD) Estimation

SOD activity was determined by the method by xanthine-xanthine oxidase system for production of superoxide flux and NBT for their production. The SOD was measured by degree of inhibition of enzyme activity after the addition of plant extracts against blank and results expressed as U/ml (33).

Catalase Activity

The catalase activity was carried using spectrophotometrically as per Atawodi method (34). The method was measure absorbance of hydrogen peroxide at 240 nm from a mixture contains serum, potassium phosphate buffer, 30 mM H₂O₂ and testing extracts against blank after 30 min. incubation (34).

Statistical Analysis

The results of *in-vivo* activity were analyzed by One-Way Analysis of variance (ANOVA) with Dunnett's T3 multiple comparison with control group using GraphPad Prism 8.

Results and Discussion

Phytochemical Analysis

The phytochemical analysis of *A. praemorsa* extracts showed presence of different phytoconstituents in them. Both the extracts have dissimilarity (Table 1), and possess sterols, terpenoids, glycosides, flavonoids, tannins, alkaloids, carbohydrates, phenols and gave negative results for amino acids. Hydroalcoholic extracts gave positive results for saponins and oils but ethyl acetate extract gave negative results.

Quantification of Phenolic and Alkaloid Contents

Both the extracts gave positive results for the presence of phenolics and alkaloids. So, their contents were quantified using standard procedures. Among two extracts hydroalcoholic extract possess more contents than ethyl acetate extract (Table 2).

In-vitro and In-vivo Antioxidant Activity

The antioxidant potential of *A. praemorsa* extracts was evaluated using different free radicals and on animals by evaluated enzyme levels with standard methods. The results of current study found the extracts possess concentration dependent potentiality on reduction of free radicals and increases the antioxidant enzymes levels in the body. Both the extracts have variation in controlling free radical and difference in percentage of inhibition and IC50 values. Among two extracts APHAE showed better activity as compared to APEAE.

Superoxide ion was a weak oxidant produced as one of the by-products during different biological metabolisms in the body (35). The produced

Table 1. Phytochemical Analysis of *Acampe praemorsa*

Name of the phytoconstituent	Name of the extract	
	Ethyl acetate	Hydro-alcoholic
Steroids	+	+
Terpenoids	+	+
Glycosides	+	+
Saponins	-	+
Flavonoids	+	+
Tannins	+	+
Carbohydrates	+	+
Alkaloids	+	+
Amino acids	-	-
Oils	-	+
Quinones	+	-
Phenols	+	+

+ = Present; - = Absent

Table 2. Phenolic and alkaloid contents of *Acampe praemorsa* extracts

Name of the Component (mg/g)	Name of the Extract	
	Ethyl Acetate	Hydroalcoholic
Total phenols	22.55±0.06	26.80±0.51
Total Alkaloids	17.68±0.25	20.59±0.22

superoxide anion in body is major precursor for production of different free radicals such as hydroxyl radicals (21). The APEAE and APHAE have showed high percentage inhibition on superoxide free radicals with 66.00±1.15 and 73.67±1.20 respectively along with ascorbic acid having 88.0±1.73 percentage inhibition (Fig. 2) on produced superoxide ions in PMS/NADH-NBT system of current study (26, 36). The IC₅₀ values were found to be 198.33 µg, 131.98 µg and 79.48 µg (Table 3).

Hydroxyl free radical scavenging activity of selected extracts was evaluated by reduction of hydroxyl radical's generation in presence of iron through Fenton reaction (27, 37, 38). The APEAE and APHAE extracts showed percentage inhibition at 320 µg on hydroxyl free radicals was 75.0±1.73 and 61.0±1.53 respectively (Fig. 3). The ethyl acetate showed more activity compared to hydro-alcoholic extract. The IC₅₀ values of APEAE, APHAE and ascorbic acid were 135.63 µg, 191.77 µg and 73.36 µg respectively (Table 3).

The DPPH free radical scavenging activity was evaluated by generating DPPH, which is a stable radical molecule can react with the proton donor from antioxidant molecules. Upon reaction color of violet will change to yellowish (39). The percentage inhibition of APEAE and APHAE extracts on reduction of DPPH free radicals was 60.0±1.15 and 80.67±1.45 respectively (Fig. 4). The hydroalcoholic extract have more activity than ethyl acetate extract. The IC₅₀ values of APEAE, APHAE extracts and ascorbic acid were found to be 204.17 µg, 115.21 µg and 73.65 µg respectively.

The hydrogen peroxide is one of major free radical which can damage cell membrane through its penetrating capability and is also precursor for hydroxyl radicals, which can initiate lipid peroxidation (40, 41). The tested extracts of *A.*

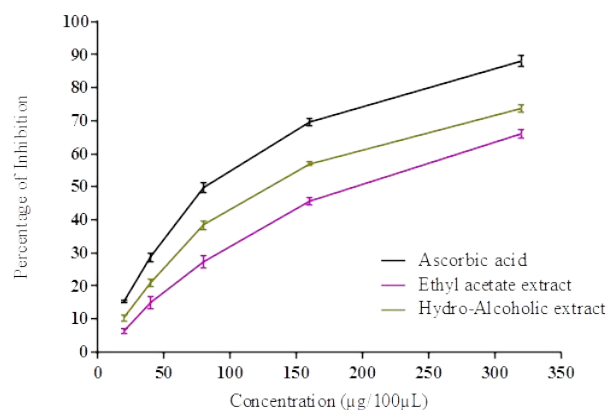


Fig. 2. In-vitro antioxidant activity of *Acampe praemorsa* extracts on superoxide free radicals.

Table 3. IC₅₀ values of *Acampe praemorsa* extracts, Ascorbic acid and Trolox on different free radicals.

Name of the extract/compound	IC ₅₀ Value (µg)				
	Name of the free radical				
	Super oxide	Hydro xyl	DPPH	H ₂ O ₂	Reducing power
APEAE	198.33	135.63	204.17	289.48	152.4
APHAE	131.98	191.77	115.21	156.77	158.23
Ascorbic acid	79.48	73.36	73.65	85.31	N/AP
Trolox	N/AP	N/AP	N/AP	N/AP	72.19

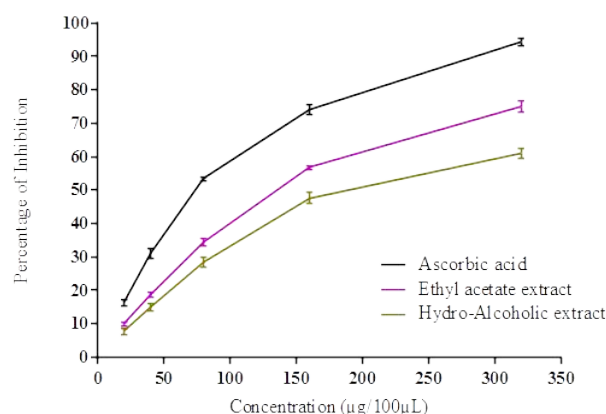


Fig. 3. In-vitro antioxidant activity of *Acampe praemorsa* extracts on hydroxyl free radicals.

praemorsa (APEAE and APHEA) showed concentration dependent percentage inhibition on produced H₂O₂ with percentage inhibition 54.00±1.73 and 70.00±1.53 at highest test concentration at 320 µg (Fig. 5). The results are compared to standard drug ascorbic acid was less and the IC₅₀ values were found to be 289.48 µg, 156.77 µg and 85.31 µg respectively (Table 3).

The reducing power assay of selected extracts (APEAE and APHEA) were carried on reduction of ferric ions to ferrous ions compared with standard drug Trolox (24). The extracts were found to possess concentration dependent activity with percentage inhibition 87.67±1.76 and 81.00±1.73 (Fig. 6). The IC₅₀ values of APEAE, APHEA and Trolox were 152.4 µg, 158.23 µg and 72.19 µg. Both the extracts had showed almost equal activity of reducing power (Table 3).

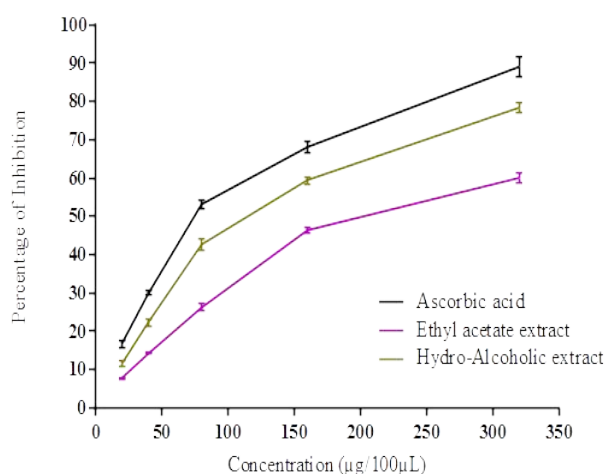


Fig. 4. *In-vitro* antioxidant activity of *Acampe praemorsa* extracts on DPPH free radicals.

The results of *in-vitro* antioxidant activity confirm presence of different antioxidant molecules in the selected plant extracts. So, *in-vivo* antioxidant activity studies were carried out evaluating oxidative enzymes

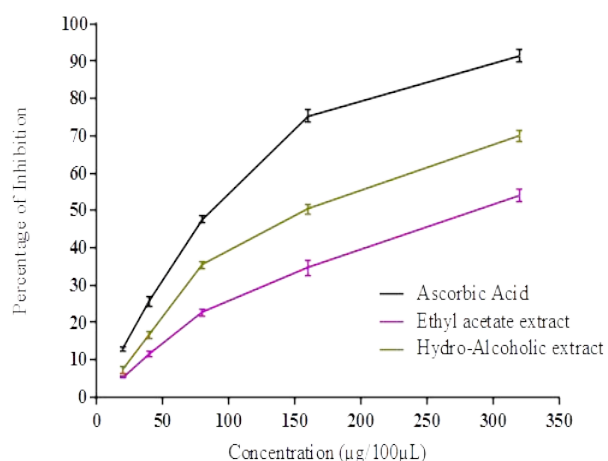


Fig. 5. *In-vitro* antioxidant activity of *Acampe praemorsa* extracts on hydrogen peroxide.

levels such as catalase, superoxide dismutase and malonaldehyde compound by lipid peroxidation. Before, proceeding the *in-vivo* study, the extracts were tested for toxicity and are found to be safe at testing or higher dosages and no physical, psychological and mortalities were observed.

The enzymatic levels of catalase, superoxide dismutase and malonaldehyde compound indicate the oxidative damage in the body through reactive oxygen species/free radicals. The catalase is a heme containing enzyme that can defend the cells in the body from different hydrogen peroxide by converting them into water and molecular oxygen (31, 42). SOD is a major enzyme that can dismutate the superoxide radicals into water and molecular oxygen (43). The results of current study in estimation of catalase SOD show that there is increase in levels of catalase and SOD than control group (Table 4) indicates the tested

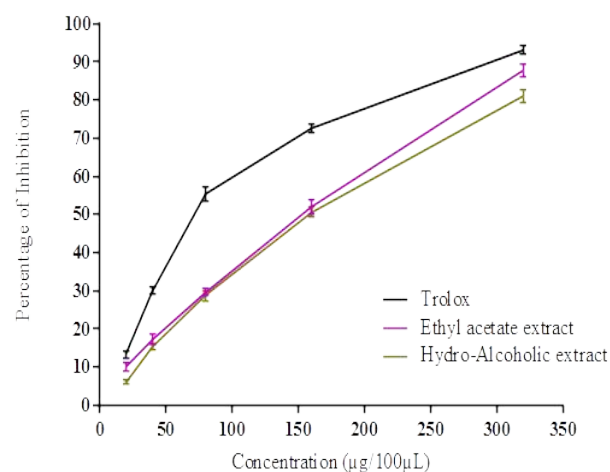


Fig. 6. *In-vitro* antioxidant activity of *Acampe praemorsa* extracts using reducing power assay.

extracts effectively increase levels of them and reduced the free radical's production.

Malonaldehyde (MDA) is a genotoxic compound produced in the body due to increased levels of lipid peroxidation (44). Lipid peroxidation is major damage in body due to the over production of free radical and reaction with cellular membrane and cause damage to deoxy ribonucleic acid (DNA) and changes in activities of membrane bound enzymes in different organs of the body (45). So, the levels of TBARS as production of MDA can indicate the controlling of free radical in the body. In the current study, level of MDA is less compared to the control group (Table 4) indicate that the extracts effectively reduce free radicals in the body and controlled the lipid peroxidation. The extracts of *A. praemorsa* exhibited concentration dependent control on enzymatic levels in the body in different groups. The hydroalcoholic extract at 400 mg/kg showed better activity compared to ethyl acetate extract.

The present study was aimed for phytochemical analysis and evaluation of antioxidant activity of *A. praemorsa* extracts. The results show that, there was variation in the phytochemical constituents in them and difference in controlling the free radicals and enzyme levels in the body. The researchers around world are successfully reporting biologically active compounds from natural resources (46, 47). Among them medicinal plants have been playing important role (10). In recent times, many researches have been reported biological activities and bioactive molecules from different medicinal plants (5, 48). In the current study, the phytochemical analysis of *A. praemorsa* extracts showed presence of bioactive molecules in them such as alkaloids, phenols, steroids etc. There were earlier reports on different medicinal plants containing alkaloids (49, 50) and phenols (51) with antioxidant potentiality and the results of present study confirms that *A. praemorsa* extracts had reduction of free radicals' generation and controlling of oxidative enzymes in the body. Among two extracts, hydro-alcoholic extracts have more phenolic and alkaloid contents as well as it had showed more potentiality against free radicals and enzyme controlling.

Table 4. Enzymatic levels in different groups due to the effect of *Acampe praemorsa* extracts at different doses.

Name of the extract/compound	Name of the enzyme/compound		
	Catalase ($\mu\text{mol/mg protein}$)	MDA (nmol/mg protein)	SOD (unit/mL)
Control	20.83 \pm 0.95	0.103 \pm 0.03	11.05 \pm 0.28
APEAE 100mg/Kg	24.67 \pm 0.71 ^{ns}	0.104 \pm 0.001 ^{ns}	14.73 \pm 0.17
APEAE 200mg/Kg	40.67 \pm 0.88*	0.085 \pm 0.002*	18.87 \pm 0.33
APEAE 400mg/Kg	84.5 \pm 1.84*	0.063 \pm 0.001*	25.08 \pm 0.26**
APHAE 100mg/Kg	29.17 \pm 0.48 ^{ns}	0.099 \pm 0.001 ^{ns}	15.99 \pm 0.06
APHAE 200mg/Kg	47.17 \pm 1.30*	0.08 \pm 0.001*	21.7 \pm 0.40
APHAE 400mg/Kg	95.67 \pm 1.20**	0.061 \pm 0.001*	28.07 \pm 0.65**

ns=Non-significant; *= $P < 0.05$; ** $P < 0.01$

Conclusion

The present study was aimed to the evaluation of phytochemicals and antioxidant activity of *Acampe praemorsa* (Roxb.) Blatt. & McCann extracts. The results of current study provide the evidence that *A. praemorsa* have different phytoconstituents in them and is having potent antioxidant activity. The extracts showed concentration dependent activity and hydroalcoholic extracts have more activity than ethyl acetate extracts and also phenolic, alkaloids contents. The variation in their activity may be due to the presence of divergence in different phytochemical compounds or their synergistic effect. In conclusion, the study provides phytochemical information of *A. praemorsa* and scientific evidence of its antioxidant activity and its folklore medicinal use. Further research is required to isolate the pure bioactive compounds and evaluate their biological activities.

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Authors' contributions

PK and KR designed current study, carried experimental work and data analysis. All authors, PK, KR, RR, KU and YV contributed in preparation of manuscript and drafting it.

Conflict of interests

The authors have none to provide.

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