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Phytochemical screening, antimicrobial, anti-inflammatory and anti-cancerous activities of ethanol and hexane extracts of *Urochloa ramosa* (L.) T.Q. Nguyen

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Abstract

Urochloa ramosa (L.) T.Q. Nguyen is known for its environmental benefits such as stabilization and reclamation of polluted soils, in agriculture to control root-knot nematodes infecting crops, in medicine to treat cardiovascular diseases, duodenal ulcer, hyperglycemia, nephritis and snake bites. Qualitative analyses of phytochemicals in ethanol and hexane extracts were performed by standard methods. *In vitro* anti-microbial assay was performed against gram positive bacteria viz., *Bacillus subtilis* and *Staphylococcus aureus,* Gram negative bacteria viz*., Pseudomonas aeruginosa* and *Escherichia coli* and fungus *Candida albicans* by disc diffusion method. Hexane extract of *Urochloa ramosa* was found to be 70% effective against *Candida albicans* indicating potent antifungal property. *In vitro* anti-inflammatory activity was performed by albumin denaturation assay, proteinase inhibition activity assay and membrane stabilization assay using various concentrations of extracts with Aspirin and Diclofenac sodium as standard. Heat induced protein denaturation was considerably prevented by ethanol and hexane extract at concentrations between 200-500 µg/ml resulting in 60 and 62% inhibition respectively. Heat induced haemolysis of erythrocyte was remarkably inhibited (59 and 68 % respectively) at concentration of 500 µg/ml. 62 and 65 % Hypotonicity induced haemolysis was also inhibited between concentration of 300-500 µg/ml in both extracts respectively. Inhibition of formation of new blood vessels by Chorioallantoic membrane (CAM) assay proved anti-angiogenic effects of extracts. Purification, characterization and structural elucidation of bioactive molecules present in ethanol and hexane extracts are needed to be explored further for assessment of better biological activites than crude extract.

Keywords

Antiangiogenic activity, anti-inflammatory activity, antimicrobial activity, Chorioallantoic membrane (CAM), phytochemicals, *Urochloa ramosa*

Introduction

Plants are rich source of bioactive phytochemicals as they serve as raw materials for the manufacture and development of drugs which offer profound benefits for the treatment of inflammatory and infectious diseases without many side effects and also they offer a more affordable treatment compared to chemically synthesized drugs with potential toxicity (1).

Phytochemicals acts as components of defense system that not only protect plants from stress conditions but also these phyto- metabolites (primary and secondary metabolites) can also be used as herbal drugs by humans, as they act as natural antioxidants thereby preventing the risk of Cancer development (2).

Invasiveness and toxigenic qualities of pathogenic microorganisms has led to the spread of many infectious diseases. In order to control the spread of pathogens, modern medicines rely upon the use of synthetic antibiotics (3). Unscientific usage of broad spectrum of antibiotic not only causes severe oxidative stress in human beings but also it has lead to the development of multidrug resistant pathogens for which the need of the hour is to depend on herbal medicines that will enable preventing infectious microbial diseases. In the present work, both antibacterial and antifungal activities of ethanol and hexane extracts were assessed by disc diffusion method (4).

At the cellular level certain physiological processes viz. protein denaturation, membrane alteration and increased vascular permeability constitutes a complex process called inflammation. It is a defensive counter attack by the body which is characterized by edema, heat, swelling and redness. There will be release of certain biochemical active components when the cells in the tissue are injured. This leads to a cascade of events that elicit inflammatory response, also involving immune competent cells leading to simultaneous healing of damaged tissues (5). Serine proteinase is abundantly localized in lysosomes of neutrophils. Leukocyte proteinase is responsible for tissue damage during inflammatory reactions and this can be prevented by supplying significant level of proteinase enzyme inhibitors. Prevention and management of inflammation related diseases can be done by the usage of bioactive principles from medicinal plants among the sick population who are relying upon synthetic drugs with deleterious side effects.

Cancer which is mainly caused by oxidative stress is a severe human disease with very high mortality rate in the world every year. Oxidants cause haemolysis. RBC's damage caused by free radicals can be prevented by the use of natural antioxidants present in plant extracts (6). Extravasation of tumor cells occur by utilization of leukocytes as they over produce macrophage migration inhibitory factor (MIF) which is a type of cytokine over expressed in many types of cancer correlating with tumor aggressiveness and poor patient outcomes. Bioactive principles such as alkaloids, tannins, flavonoids, anthocyanins etc., in plants are known to deplete the concentration of MIF causing delay in tumor growth and metastasis (7).

Emergence and development of new blood vessels from pre-existing vasculature is known as angiogenesis. This process is brought about by TNF-α & β, PDGF, HGF and VEGF where VEGF is a prime regulator of normal and tumor angiogenesis (8). Phytochemicals in plants can be used as drugs in anti-angiogenesis therapy which focus on preventing the formation of new blood vessels around tumor. Anti-angiogenesis causes ablation and destruction of tumor vessels; it is less toxic and also prevents the spread of cancer to other organs when compared to chemotherapy which suppress body's immune system (9). Hence, Chick chorioallantoic assay which is one of the routinely used, conventional histological procedures was used to study angiogenesis and it was performed using ethanol and hexane extracts of *U. ramosa* (10).

Urochloa ramosa (L.) T.Q. Nguyen, commonly known as 'Brown top millet' is an annual weedy grass in Poaceae. In India, it is often cultivated for grains and as a forage crop. It is also grown as a nurse crop especially on slopes, which can assist in the establishment of a perennial crop. The ash of the leaves is to treat cardiovascular diseases, duodenal ulcer, hyperglycemia, nephritis and snake bites by traditional healers. It is used in environmental remediation projects, as it accumulates significant amounts of zinc and lead from the soil and hence it can be a potential source for easy removal of toxic minerals.

The plant also possess antimicrobial, antioxidant, anti-inflammatory and anti-angiogenic properties.

Materials and Methods

All reagents and solvents were of analytical grade used in the present study. Fertilized hen's eggs were collected from Indian Veterinary Research Institute (IVRI), Bangalore. *Urochloa ramosa* (TSN-523031) was collected from fields of Mysore, Karnataka state, India. The plant was collected from its natural habitat and authenticated by an Angiosperm taxonomist from University of Mysore. The leaves were thoroughly washed, dried under shade, fine powdered, sieved and stored till further use in air tight container. 100 g of leaves were weighed and subjected to sequential extraction using solvents like hexane, chloroform, ethyl acetate, acetone ethanol and methanol (non polar to polar) taken in Soxhlet apparatus. Hexane extract and ethanol extracts were filtered and used for screening of phytochemicals and assessment of biological activities like antimicrobial, anti-inflammatory and anti-cancerous activities.

Phytochemical Analysis

The hexane and ethanol extracts of the plant were subjected to preliminary phytochemical analysis to trace the presence of phytochemicals like Carbohydrates, Tannins, Saponins, Alkaloids, Flavonoids, Glycosides, Quinones, Phenols, Terpenoids, Cardiac glycosides, Amino acids, Coumarins, Anthraquinones, Steroids, Lignin, Phlobatannins, Anthocyanin, Balsams, Volatile oils and Fatty acids (Table 1) as per the standard methods (11-13).

Antimicrobial activity

Gram-positive bacteria -*Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538) and Gram-negative bacteria - *Pseudomonas aeruginosa* (ATCC 9027), *E. coli* (ATCC 25922) and fungi *Candida albicans* (ATCC 3147) were used for the determination of antimicrobial potential of ethanol and hexane extract of *U. ramosa* by disc diffusion method. 0.1 g of extract was transferred to 100 ml of ethanol and hexane and dissolved to form a final concentration of 1 mg/ml. This forms stock solution of the extract. Extract

Table 1. Qualitative Phytochemical tests

Phytochemicals	Test	Observation
Carbohydrates	1ml extract + 1ml Molish's reagent+ 2 drops of sulphuric acid	Purple colour
Tannins	1ml extract + 2ml 5% ferric chloride	Dark blue colour
Saponins	1ml extract + 1ml distilled water+ shaken well	Foam formation
Alkaloids	1ml extract + 1ml HCl + 2 drops of Mayer's reagent	Green colour
Flavanoids	1ml extract + 1ml 2N sodium hydroxide	Yellow colour
Glycosides	1ml extract + 2ml chloroform + 1ml 10% ammonia solution	Pink colour
Quinones	1ml extract + 1 ml sulphuric acid	Red colour
Phenols	1ml extract + 1 ml distilled water + 4 drops 10% ferric chloride	Blue/green colour
Terpenoids	1ml extract + 1 ml chloroform + 1ml sulphuric acid	Reddish brown colour
Cardiac glycosides	1ml extract + 1 ml glacial acetic acid + 4 drops of 10% ferric chloride + 1 ml sulphuric acid	Brown colour
Amino acids	1ml extract + 2 drops of 0.2% ninhydrin+ heat	Pink/purple colour
Coumarins	1ml extract + 1 ml 10% sodium hydroxide	Yellow colour
Anthraquinones	1ml extract + 4 drops 10% ammonia solution	Pink colour
Steroids	1ml extract + 1ml chloroform+ 4 drops sulphuric acid	Brown ring formation
Lignin	1ml extract + 1ml Phloroglucinol-HCl	Red/violet colour
Phlobatanins	1ml extract + 4 drops 2% HCl	Red colour precipitate
Anthocyanins	1ml extract + 1ml 2N sodium hydroxide + heat	Bluish green colour
Balsams	1ml extract + 1ml 90% ethanol + alcoholic ferric chloride	Dark green colour
Volatile oils	1ml extract + 4 drops dilute HCl	White precipitate
Fatty acids	1ml extract + 2ml ether + mixed, evaporated on filter paper	Transparency on filter paper

of 20 µl of various concentrations - 50, 100, 200, 400 µg/mL dissolved in Dimethyl formamide were impregnated onto 6 mm diameter sterile discs. Dimethyl formamide loaded disc was considered as negative control. Sterile discs containing various concentrations of extracts were layer onto the lawn of microbial colonies in the petri plate; it was followed by incubation at 37 ˚C for 24 hrs to determine the zone of inhibition in mm. Gentamycin and Flucanazole were used as positive control (14-16).

In vitro anti-inflammatory activity

a. Albumin denaturation inhibition assay

Different concentrations (100 - 500 µg/ml) of ethanol and hexane extracts were mixed with 1% of bovine albumin and the aliquots were incubated at 37 °C for 20 min. The samples were heated to 51 °C for 20 min. and cooled. Turbidity of the sample was read at 660 nm using UV Vis Spectrophotometer. 100 µg/ml Aspirin was used as standard drug (17, 18). Inhibition of denaturation of albumin protein in terms of percentage was evaluated by

% inhibition = (Abs Control – Abs Sample) X 100/ Abs control.

b. Anti-proteinase activity

Aliquots having 100 - 500 µg/ml concentrations of ethanol and hexane extracts of *U. ramosa* were mixed with 0.05 mg Trypsin followed by addition of 1 ml 20 mM Tris HCL buffer with pH- 7.4. The sample reaction mixture was then incubated at 37 °C for 5 min., followed by the addition of 1 ml 8% casein. The aliquots were again incubated for 20 min. To stop the reaction 2 ml of 70% perchloric acid was added. There was appearance of cloudy white precipitate that was centrifuged and the absorbance of supernatant was read at 210 nm against the buffer taken as blank solution. Aspirin was used as standard drug for comparison (19).

The % of proteinase inhibitory activity was calculated as

% inhibition = (Abs control –Abs sample) X 100/ Abs control.

c. Stabilization of RBC membrane

Preparation of Red Blood cells (RBCs) suspension

Non steroidal anti-inflammatory drug free 5 ml blood was collected from healthy volunteer. It was centrifuged at 3000 rpm for 5 min at room temperature. Later, the blood was washed with (0.9% NaCl) saline. After centrifugation blood was reconstituted as 10% v/ v suspension using isotonic sodium phosphate buffer (10 mM, pH- 7.4) (20).

d. Heat induced haemolysis

Various concentrations (100-500 µg/ml) of ethanol and hexane extracts of *U. ramosa* were mixed with 1 ml of 10% erythrocyte suspension. Saline (0.9% Sodium chloride) was used as control and standard drug Aspirin was used for comparative studies. All the aliquots were incubated at high temperature of 56 °C in water bath for 30 min. The reaction mixtures were cooled, centrifuged at 2500 rpm for 5 min. and the absorbance of supernatant was read at 560 nm (21, 22). Percentage inhibition of haemolysis was calculated by

% inhibition = (Abs control – Abs sample) X 100/ Abs control.

e. Haemolysis induced by hypotonic solution

In order to assess the herbal potency on haemolysis induced by hypotonic solution, 0.5 ml of 10 % erythrocyte suspension, 1 ml of phosphate buffer and 2 ml of hyposaline (0.26 %) were mixed with different concentrations (100 -500 µg/ml) of both the extracts. Standard drug Diclofenac sodium was used for comparison. The assay mixtures were incubated at 37 °C for 30 min. to induce breakage of RBC membrane. The reaction mixtures were centrifuged at 3000 rpm for 5 min. Supernatant was decanted and the absorbance was recorded at 560 nm to estimate percentage of inhibition of hypotonicity induced haemolysis (21). This can be calculated by the following equation

% protection = 100- (OD sample/OD control) x 100.

Assessment of Anticancerous property

a. Anti-hemolytic activity

The principle of this assay is that H_2O_2 induces oxidative stress on lipids in human blood erythrocytes. H_2O_2 induced membrane damage is due to its chain oxidation activity on lipids and proteins which eventually lead to the loss of membrane organization and integrity, finally leading to complete haemolysis (21, 22).

Aliquots containing (100-500 μg/ml) concentration of extracts were mixed with 1 ml of RBC suspension and 0.5 ml of H_2O_2 solution. The reaction mixture was incubated for 20 min at room temperature. The samples were centrifuged at 3000 rpm for 10 min. Supernatant was collected and absorbance was recorded at 540 nm (23). Phosphate buffer was taken as blank. Percentage inhibition of haemolysis was calculated by the following:

Hemolysis (%) = (Sample abs/ control abs) X 100

Protection (%) = 100 - % hemolysis

b. Leucocyte migration assay

Whole blood was freshly collected from a healthy volunteer. It was centrifuged at 1500 rpm for 15 min at room temperature. White buffy layer of leucocytes were separately collected. 1% solution of agar was poured on to glass plates, allowed to solidify and two wells were punched out (opposite to one another) on each plate. 250 μg/ml of both ethanol and hexane extracts were loaded into one well and the collected leucocytes were loaded into the other separately. This creates a chemical gradient for migration of leucocytes. Migrated cells were observed under light microscope after 24 hr incubation at 37 °C (24-26).

c. Chick Chorioallantoic Membrane Assay

The CAM assay was considered to study the effect ethanol and hexane extracts of *U. ramosa* on angiogenesis. Fertilized chicken eggs were collected from IVRI Bangalore, Karnataka. Surface sterilization of the eggs was performed using 70% ethanol. Eggs were kept inside the fan assisted humidifier and incubated at 37 °C for 3 days. Eggs were rotated frequently for uniform incubation. After 3 days of incubation a small opening was made on the narrow end of the egg with the help of sterile needle and scalpel to form a window. 100 μg of ethanol and hexane extracts were loaded onto the sterile filter paper disc and it was then placed inside the egg next to the blood vessels. Sterile adhesive tape was used to seal the window. The window was covered with transparent plastic sterile sheet and the eggs were again transferred to incubator for another 3 days. After incubation for 6-8 days the adhesive tape was removed carefully and the eggs were checked for anti-angiogenesis effect i.e.; blood coagulation or breakage in blood vessels by the extracts. The above protocol was carried out in laminar air flow hood (27, 28).

Statistical analysis

Values are means of three independent replicates (n = 3) and ± indicates standard deviation. Means followed by the same letter(s) within the same column are not significantly ($p \leq 0.05$) different according to Tukey's HSD.

Results and Discussion

Qualitative analysis of phytochemicals

The phytochemicals found in ethanol and hexane extracts are presented in Table 2.

Antimicrobial activity

Antimicrobial activity of two extracts of *U. ramosa* was determined by evaluation of zone of inhibition formed around the discs. Among five different concentrations, 400 μg/ml showed maximum activity against all the microbes viz.; *Bacillus subtilis* (8.0 mm and 9.0 mm), *Staphylococcus aureus* (5.0 mm and 7.0 mm), *Pseudomonas aeruginosa* (6.0 mm and 8.0 mm), *E. coli* (8.0 mm and 11.0 mm) *and Candida albicans* (12 mm and 13 mm) respectively. Formation of zone of inhibition was recorded in *Bacillus subtilis* and *Candida albicans* at all the concentrations in both ethanol and hexane extracts. There was no formation of zone of inhibition at 50 μg/ml concentration in *Staphylococcus aureus, Pseudomonas aeruginosa* and *E. coli* in both the extracts. It was observed that ethanol and hexane extracts were more effective against *Bacillus subtilis* and *Candida albicans* and in particular there was more growth retardation and arrest in *Candida albicans* indicating potent antifungal property than antibacterial property of hexane extract (Table 3; Fig. 1).

Table 2. Details of phytochemicals found in ethanol and hexane extracts of *U. ramosa*

Phytocompounds	Ethanol extract	Hexane extract
Carbohydrates	+	-
Tannins	+	-
Saponins	+	+
Alkaloids	-	-
Flavonoids	+	-
Glycosides	-	+
Quinones	-	+
Phenols	+	-
Terpenoids	+	+
Cardiac glycosides	-	+
Amino acids	+	-
Coumarins	-	+
Anthraquinones	-	-
Steroids	-	+
Lignin	+	-
Phlobatannins	-	+
Anthocyanin	+	-
Balsams	+	-
Volatile oils	-	+
Fatty acids	-	+

Note: + indicates presence and - indicates absence of phytoconstituents

Anti-inflammatory activity

a. Inhibition of albumin denaturation

During inflammatory mechanism proteins lose their molecular structure and become non-functional (denaturation) (17). It is evident that maximum inhibition was recorded at 500 μg/ml in both extracts with 60 and 62% inhibition of albumin denaturation respectively in

comparison to standard aspirin with inhibitory action at 100 µg/ml (Table 4).

Table 3. Antimicrobial activity of ethanol and hexane extract of *U. ramosa* - Zone of inhibition (mm) induced by plant extract by disc diffusion method

Test Pathogens	Extract	Concentration (µg)				Gentamycin	Flucanazole
		50	100	200	400		
Bacillus subtilis	EUR	2.26 ± 0 .30^{b}	5.00 ± 0.20^{a}	7.20 ± 0.20^{a}	8.06 ± 0.20^{c}	18.00 ± 0.00^{a}	-
	HUR	3.86 ± 0.11^{a}	5.10 ± 0.10^{a}	6.90 ± 0.10^{b}	9.00 ± 0.20^{b}		-
Staphylococcus aureus	EUR	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	2.06 ± 0.05^{f}	4.96 ± 0.05^{f}	17.86 ± 0.11^{a}	-
	HUR	0.00 ± 0.00^{c}	3.03 ± 0.15^{b}	4.96 ± 0.05^{d}	7.00 ± 0.00^{d}		-
Pseudomonas aeru-ginosa	EUR	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	2.03 ± 0.05^{f}	6.00 ± 0.00^{e}	18.00 ± 0.00^{a}	-
	HUR	0.00 ± 0.00^{c}	2.96 ± 0.05^{b}	5.96 ± 0.05^{c}	8.06 ± 0.05^{c}		-
E. coli	EUR	0.00 ± 0.00^{c}	1.86 ± 0.11^{c}	3.96 ± 0.05^{e}	7.96 ± 0.15^{c}	13.96 ± 0.05^{b}	-
	HUR	0.00 ± 0.00^{c}	1.93 ± 0.11^{c}	4.93 ± 0.11^{d}	10.63 ± 0.55^{a}		-
Candida albicans	EUR	3.30 ± 0.20^{b}	6.00 ± 0.30^{b}	8.43 ± 0.45^{b}	12.03 ± 0.25^{a}	-	18.83 ± 0.28^{a}
	HUR	4.96 ± 0.25^{a}	8.03 ± 0.15^{a}	10.06 ± 0.20^{a}	12.93 ± 0.11^{a}	-	

EUR- Ethanol extract of *Urochloa ramosa*, HUR- Hexane extract of *Urochloa ramosa* . Values are means of three independent replicates (n = 3) and ± indicates standard deviation. Means followed by the same letter(s) within the same column are not significantly ($p \leq 0.05$) different according to Tukey's HSD.

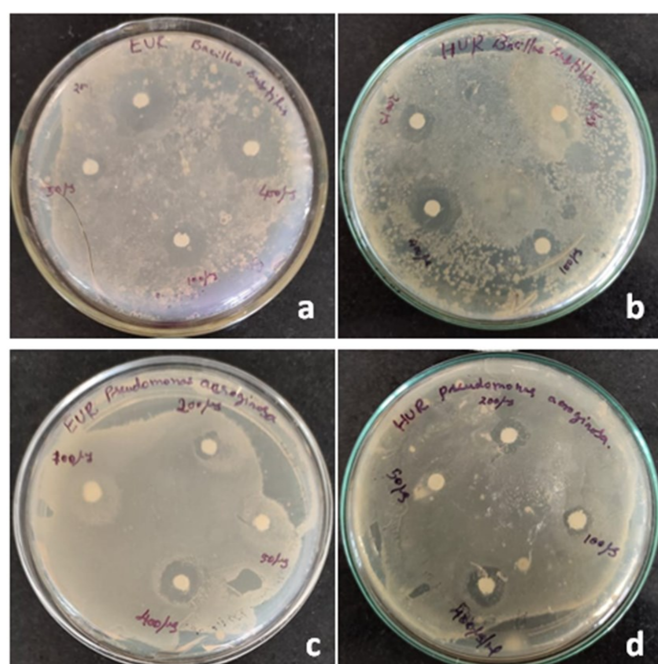


Fig. 1. The inhibition zone (mm) of ethanol (a & c) and hexane (b &d) extracts of *U. ramosa* on *Bacillus subtilis* and *Pseudomonas aeruginosa*

b. Proteinase Inhibitory Action

Ethanol and hexane extracts exhibited sufficient anti-proteinase activity at different concentrations as represented in table: 5. Maximum proteinase action inhibition was recorded at 500 µg/ml concentration and it was 64 and 67% in ethanol and hexane extract respectively (Table 5).

c. Membrane stabilization

Stabilization of membrane is very important to prevent elicitation of inflammatory response during the tissue injury. *In-vitro* anti-inflammatory activity proved to inhibit distortion of RBC membrane which will implies that the extract will also stabilize lysosomal membrane as RBC membrane is analogous to lysosomal membrane. Extracellular release of constituents of lysosomes from neutrophils (certain lytic substances and proteases) bring about tissue damage indicating development of acute or chronic inflammation (19).

d. Heat Induced Haemolysis

Table 4. Effect of ethanol and hexane extract of *U. ramosa* on heat induced protein denaturation

Concentration µg/ml)	*U. ramose* extract	Absorbance at 660 nm	% Inhibition
100	EUR	0.52 ± 0.004b	3.66 ± 0.57h
	HUR	0.41 ± 0.000d	13.33 ± 1.15g
200	EUR	0.48 ± 0.003c	11.33 ± 0.57g
	HUR	0.36 ± 0.004f	2.33 ± 0.57hi
300	EUR	0.40 ± 0.000e	25.66 ± 0.57f
	HUR	0.28 ± 0.004h	41.66 ± 1.15e
400	EUR	0.32 ± 0.003g	42.00 ± 1.73e
	HUR	0.22 ± 0.000i	54.66 ± 1.15d
500	EUR	0.22 ± 0.001i	60.66 ± 1.15c
	HUR	0.18 ± 0.003j	63.00 ± 1.00b
Control	EUR	0.54 ± 0.000a	0.00 ± 0.00j
	HUR	0.48 ± 0.000c	0.00 ± 0.00j
Aspirin (100)	-	0.12 ± 0.001k	78.00 ± 1.00a

EUR- Ethanol extract of *Urochloa ramosa*, HUR- Hexane extract of *Urochloa ramose.* Values are means of three independent replicates (n = 3) and ± indicates standard deviation. Means followed by the same letter(s) within the same column are not significantly ($p \leq 0.05$) different according to Tukey's HSD

Table 5. Effect of ethanol & Hexane extract of *U. ramosa* on proteinase inhibitory action

Concentration (µg/ml)	*U. ramosa* extract	Absorbance at 210 nm	% Inhibition
100	EUR	0.36 ± 0.000c	23.33 ± 0.57g
	HUR	0.39 ± 0.000b	16.66 ± 1.15h
200	EUR	0.31 ± 0.004d	34.66 ± 1.15f
	HUR	0.31 ± 0.006d	35.66 ± 1.15f
300	EUR	0.28 ± 0.003e	41.00 ± 1.73e
	HUR	0.26 ± 0.003f	42.33 ± 1.15e
400	EUR	0.21 ± 0.004g	55.33 ± 0.57d
	HUR	0.21 ± 0.001g	54.66 ± 1.15d
500	EUR	0.17 ± 0.001h	63.33 ± 1.15c
	HUR	0.15 ± 0.001i	67.00 ± 0.00b
Control	EUR	0.47 ± 0.004a	0.00 ± 0.00i
	HUR	0.46 ± 0.004a	0.00 ± 0.00i
Aspirin (100)	-	0.09 ± 0.004j	81.00 ± 1.00a

EUR- Ethanol extract of *Urochloa ramosa*, HUR- Hexane extract of *Urochloa ramosa* , Values are means of three independent replicates (n = 3) and ± indicates standard deviation. Means followed by the same letter(s) within the same column are not significantly ($p \leq 0.05$) different according to Tukey's HSD

At the concentration of 500 µg/ml the extract recorded inhibition of 15 and 68% of heat induced haemolysis respectively. But aspirin being a standard synthetic drug proved to be more effective offering 74% inhibition at 100 µg/ml concentration when compared to that of the plant extracts (Table 6).

e. Haemolysis induced by hypotonic solution

There was significant inhibition even in haemolysis induced by hypotonic solution, maximum protection was observed at 500 µg/ml with 62 and 65% inhibition in EUR and HUR respectively, whereas Diclofenac sodium at a concentration of 100 µg/ml offered 79% protection (Table 7).

Assessment of Anticancerous properties

a. Anti-hemolysis assay

Marked reduction of haemolysis was observed when the extracts were treated with RBC along with the toxicant H_2O_2. At the concentration of 500 µg/ml ethanol extract showed only 51% inhibition but Hexane extract showed 79% inhibition of anti-hemolytic activity. Diclofenac sodium (100 µg/ml) showed 85% inhibition of haemolysis. Antihaemolytic activity may due to the presence of secondary metabolites in the extracts. Phytochemicals act as very good electron and hydrogen atom donors; they can also terminate radical chain reactions by scavenging free radicals and reactive oxygen species (Table 8).

Table 6. Effect of ethanol and hexane extract of *U. ramosa* on heat induced haemolysis

Concentration (µg/ml)	*U. ramosa* extract	Absorbance at 560 nm	% Inhibition
100	EUR	0.32 ± 0.001c	22.33 ± 0.57k
	HUR	0.28 ± 0.004d	27.33 ± 0.57j
200	EUR	0.28 ± 0.006d	32.66 ± 1.15i
	HUR	0.24 ± 0.003f	36.66 ± 1.15h
300	EUR	0.25 ± 0.004e	39.33 ± 0.57g
	HUR	0.22 ± 0.004g	42.33 ± 0.57f
400	EUR	0.21 ± 0.000h	48.33 ± 1.15e
	HUR	0.18 ± 0.005i	52.66 ± 0.57d
500	EUR	0.17 ± 0.003i	59.33 ± 0.57c
	HUR	0.12 ± 0.005j	68.33 ± 0.57b
Control	EUR	0.41 ± 0.003a	0.00 ± 0.00l
	HUR	0.38 ± 0.002b	0.00 ± 0.00l
Aspirin (100)	-	0.18 ± 0.005i	74.66 ± 1.15a

EUR- Ethanol extract of *Urochloa ramosa*, HUR- Hexane extract of *Urochloa ramosa* . Values are means of three independent replicates (n = 3) and ± indicates standard deviation. Means followed by the same letter(s) within the same column are not significantly (p ≤ 0.05) different according to Tukey's HSD.

Table 7. Effect of ethanol and hexane extract of *U. ramosa* on hypotonic solution induced haemolysis

Concentration (µg/ml)	*U. ramosa* extract	Absorbance at 560 nm	% Inhibition
100	EUR	0.22 ± 0.190 cde	22.33 ± 0.57h
	HUR	0.29 ± 0.005bc	21.66 ± 0.57h
200	EUR	0.26 ± 0.006bcd	31.66 ± 0.57g
	HUR	0.22 ± 0.001cde	38.33 ± 0.57f
300	EUR	0.21 ± 0.004cde	39.33 ± 0.57f
	HUR	0.18 ± 0.004cde	49.66 ± 0.57e
400	EUR	0.19 ± 0.004cde	49.66 ± 1.15e
	HUR	0.16 ± 0.003cde	54.33 ± 0.57d
500	EUR	0.16 ± 0.004cde	58.66 ± 0.57c
	HUR	0.13 ± 0.003de	61.66 ± 0.57b
Control	EUR	0.42 ± 0.004a	0.00 ± 0.00i
	HUR	0.37 ± 0.003ab	0.00 ± 0.00i
Aspirin (100)	-	0.08 ± 0.004e	79.33 ± 0.57a

EUR- Ethanol extract of *Urochloa ramosa*, HUR- Hexane extract of *Urochloa ramosa*
Values are means of three independent replicates (n = 3) and ± indicates standard deviation. Means followed by the same letter(s) within the same column are not significantly (p ≤ 0.05) different according to Tukey's HSD

b. Leucocyte migration assay

Leukocytes and their soluble substances play a critical role during primary tumor development and metastasis. Leukocytes infiltrate inflamed tissues and release certain growth factors that cause change in the behavior of the cell and lead to the formation of tumour. Most carcinoma tissue contains subsets of leucocytes including myeloid- and lymphoid-lineage cells. Monocytes and macrophages are also leukocytes that will maintain cellular and tissue homeostasis and they also improve immunity. Macrophages and tumor cells interact directly that can lead to invasion, intravasation, extravasation and angiogenesis. Therefore, inhibition of leucocyte and macrophage movement can restrict the migration and metastasis of carcinoma cells (24, 25). 250 µg/ml of both ethanol and hexane extracts of *U. ramosa* showed maximum inhibition of cellular migration; most cells were seen to be retained at the region of loading.

Table 8. Effect of ethanol and hexane extract of *U. ramosa* on haemolysis induced by H_2O_2

Concentration (µg/ml)	*U. ramosa* extract	Absorbance at 560 nm	% Inhibition
100	EUR	0.39 ± 0.004^{b}	12.33 ± 0.575^{h}
	HUR	0.28 ± 0.004^{e}	30.66 ± 1.15^{f}
200	EUR	0.35 ± 0.003^{c}	23.66 ± 6.35^{g}
	HUR	0.22 ± 0.004^{f}	45.66 ± 1.15^{d}
300	EUR	0.31 ± 0.004^{d}	29.33 ± 0.57^{f}
	HUR	0.18 ± 0.003^{g}	55.66 ± 1.15^{c}
400	EUR	0.27 ± 0.004^{e}	37.33 ± 1.15^{e}
	HUR	0.10 ± 0.004^{h}	75.66 ± 1.15^{b}
500	EUR	0.22 ± 0.004^{f}	51.66 ± 1.15^{c}
	HUR	0.08 ± 0.004^{hi}	79.66 ± 1.15^{b}
Control	EUR	0.44 ± 0.004^{a}	0.00 ± 0.00^{i}
	HUR	0.43 ± 0.041^{a}	0.00 ± 0.00^{i}
Diclofenac	-	0.06 ± 0.004^{j}	85.66 ± 1.15^{a}

EUR- Ethanol extract of *Urochloa ramosa*, HUR- Hexane extract of *Urochloa ramosa*
Values are means of three independent replicates (n = 3) and ± indicates standard deviation.
Means followed by the same letter(s) within the same column are not significantly (p ≤ 0.05) different according to Tukey's HSD

c. CAM Assay

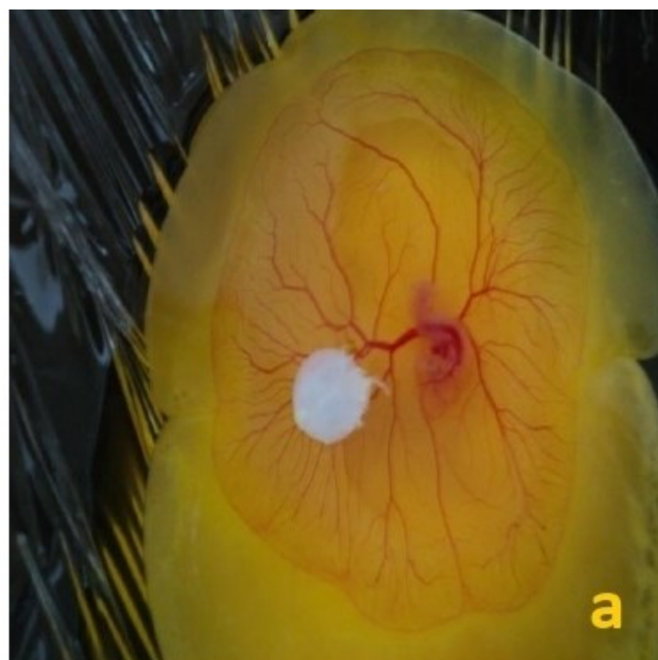
The results obtained from the present study suggest that

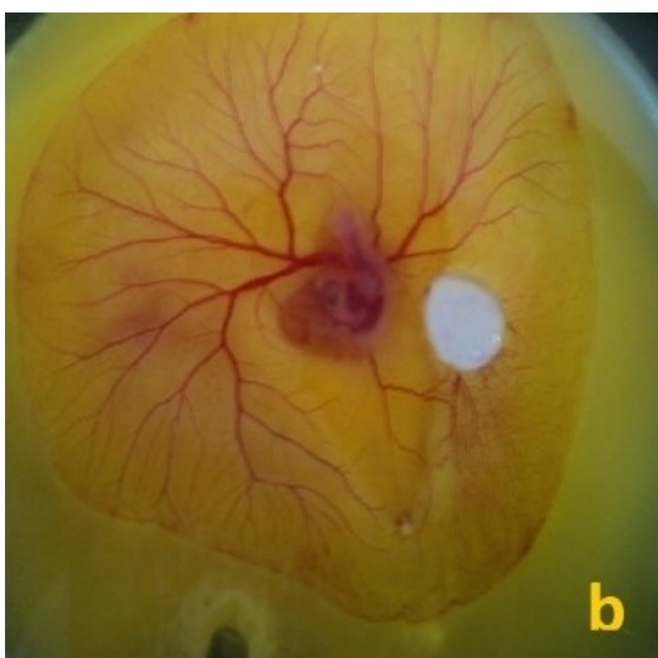
Hexane extract offered significant antiangiogenic activity (inhibition of formation of vasculature) when compared to ethanol extract with showed comparatively less antiangiogenic activity (Fig. 2).

Conclusion

Plants are backbone of sophisticated traditional medicine systems dating back to hundreds of years and persist to supply human mankind with new remedies. The use of synthetic chemical compounds has led to decline in the use of plants in contemporary medicine over years (29). Botanicals are considered as best source of medicine, as they are eco-friendly, more affordable than conventional medicine, easier to obtain than prescribed medicine, strength in immune system, fewer side effects and cost effective (30).

Upon qualitative analysis hexane extract of *U. ramosa* confirmed the presence of biologically active and medicinally important active compounds in both ethanol and hexane extracts proving potent antimicrobial, antioxidant, anti-inflammatory, anti-cholinergic, anti-angiogenic and other biological activities.





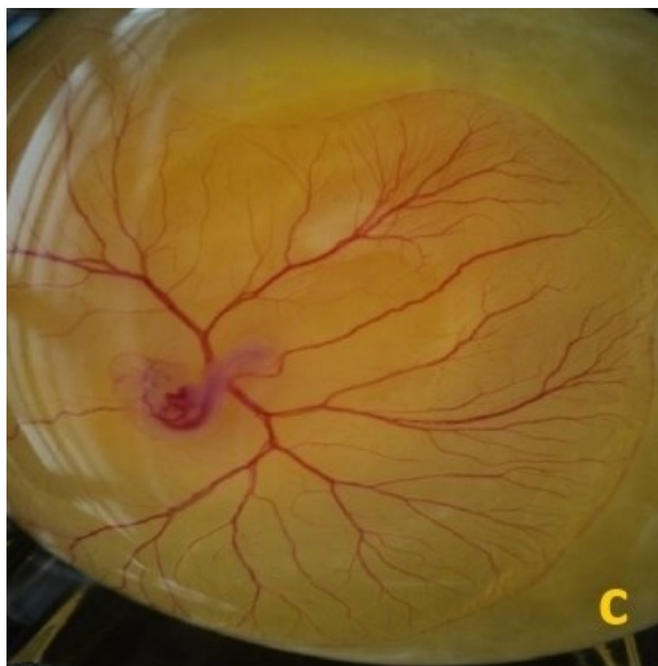


Fig. 2. Antiangiogenic effect of ethanol (a) hexane (b) extracts of *U. ramosa*

Presently use of antibiotics and other synthetic compounds is very much spread in medicine and agriculture that has led to the emergence of antibiotic resistant strains of microbes that eventually cause very serious problem in controlling infectious diseases as a result researchers are exporting new antimicrobial compounds mainly from plant source. On the basis of this antimicrobial study, it is clear that the selected plant shows a considerable activity against selected microbes. Therefore, this study indicates that *U. ramosa* may be used as potent antimicrobial drugs of natural origin.

The ethanol and hexane extracts of *U. ramosa* showed anti-proteinase activity, anti-haemolytic activity and inhibition of denaturation of proteins. These activities establish that the plant possess antiinflamatory property that might be due to the presence of certain secondary metabolites like Tannins, Saponins, Flavonoids, Glycosides, Quinones, Phenols, Terpenoids etc. Antihaemolytic assay, Leucocyte migration assay and CAM assay showed anticancerous activities of the extract. Hence, the plant extract act as an important resource for disease treatment. CAM is a standard technique used to understand angiogenesis, growth and invasiveness of tumour and for the screening of anti-tumor drugs. Recent studies show that plants having high antioxidant activity can also shoe strong antiangiogenic activity (30, 31). Many secondary metabolites like polyphenols, terpenoids and flavonoids have the ability to inhibit tumorigenesis. The occurrence of bioactive compounds in the *U. ramosa* indicates that this plant may have possible use in biomedical research. From the above result it is also evident that ethanol and hexane extract of *U. ramosa* has a promising anti-angiogenic agent and could play a vital role as a source of chemotherapeutic agent in the

treatment of tumors. It is an absolute requirement to purify the bioactive compounds form ethanol and hexane extracts so that in its purified form it can show high biological activities.

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Authors contributions

AMS, NDR and SK did the experiments, AMS, NDR, ACU and SK wrote the manuscript, AMS, NDR, ACU and SK read the manuscript and made suitable changes.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical issues: None

References

1. Amrit PS. Promising phytochemicals from Indian Medicinal plants. Ethnobotanicals leaflets. 2005; 18.
2. Doss A. Preliminary phytochemical screening of some Indian Medicinal plants. Anc Sci Life. 2009; 29:12-16.
3. Arunakumar S, Muthuselvam. Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. against clinical pathogen: World J Agril Sec. 2009;5(5):572-76.
4. Nascimento GGF, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz J Microbiol. 2000; 31(1):247-56. https://doi.org/10.1590/S1517-83822000000400003
5. Gerard JT, Sandra R. Principles of Anatomy and Physiology. Harper Collins College Publishers. 1993; 7:695.
6. Adwaid MK, Ambili N, Lokesh R, Sarveswari S. Anti-haemolytic and Antioxidant Activity of *Piper longum* Seed. Advances in Biotechnology and Microbiology. 2017;4(4):555-642. https://doi.org/10.19080/AIBM.2017.04.555642
7. Balogh KN, Templeton DJ, Cross JV. Macrophage Migration Inhibitory Factor protects cancer cells from immunogenic cell death and impairs anti-tumor immune responses. PloS one. 2018;13 (6):e0197702. https://doi.org/10.1371/journal.pone.0197702
8. Domenico R, Beatrice N, Angelo V, Luisa R, Burri HP, Valentin D. Chorioallantoic membrane capillary bed: A useful target for studying angiogenesis and anti-angiogenesis *in vivo*. The Anatomical Record. 2001;264(4):317-24. https://doi.org/10.1002/ar.10021
9. Quigley JP, Armstrong PB. Tumor cell intravasation elucidated: the chick embryo opens the window. Cell. 1998;94(3):281-84. https://doi.org/10.1016/S0092-8674(00)81470-1
10. Ribatti D., Conconi, M T, Nico B. Angiogenic response induced by acellular brain scaffolds grafted onto the chick embryo chorioallantoic membrane, Brain Res. 2003;989(1):9–15. https://doi.org/10.1016/s0006-8993(03)03225-6.
11. Igbinosa OO, Igbinosa EO, Aiyegoro OA. Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn.), African Journal of Pharmacy and Pharmacology. 2009;3(2):58-62. https://doi.org/10.1.1.561.1288
12. Harbone JB. Phytochemicals methods, London. Chapman and Hill, 1973.
13. Peach K, Tracey MV. Modern methods of plant analysis, Springer Verlag, Berlin, 1956; 3.
14. Mothana RAA, Lindequist U. Antimicrobial activity of some medicinal plants of the island Soqotra. J of Ethnopharmacology. 2005;(96)1-2:177–81. https://doi.org10.1016/j.jep.2004.09.006
15. Selvamohan T, Ramadas V, Kishore SS. Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. Advances in Applied Science Research. 2012;3 (5):3374–81.
16. Wigmore SM, Naiker M, Bean DC. Antimicrobial activity of extracts from native plants of temperate Australia. Pharmacognosy Communications. 2016;6:80-84. https://doi.org/10.5530/pc.2016.2.5
17. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. J of Pharma Pharmacol. 1968;20:169-73. https://doi.org 10.1111/j.2042-7158.1968.tb09718.x
18. Sakat S, Juvekar AR, Gambhire MN. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int J of Pharma and Pharmacological Sciences. 2010;2 (1):146-55.
19. Oyedepo OO and Femurewa AJ. Anti-protease and membrane stabilizing activities of extracts of *Fagra zanthoxiloides*, Olax *subscorpioides* and *Tetrapleura tetraptera*. Int J of Pharmacong. 1995;33:65-69. https://doi.org/10.3109/13880209509088150
20. Sadique J, Al-Rqobahs WA, Bughaith, EIGindi AR. The bioactivity of certain medicinal plants on the stabilization of RBS membrane system. Fitoterapia.1989;60:525-32.
21. Shenoy S, Shwetha K, Prabhu K, Maradi R, Bairy KL, Shanbhag T. Evaluation of antiinflammatory activity of *Tephrosia purpurea* in rats. Asian Pac J Trop Med. 2010;3(3):193-95. https://doi.org/10.1016/S1995-7645(10)60007-7
22. Gandhidasan R, Thamaraichelvan A, Baburaj S. Anti inflammatory action of *Lannea coromandelica* by HRBC membrane stabilization. Fitoterapia 1991;62(1):81-83.
23. Shobana S, Vidhya R. Evaluation of in vitro hemolytic activity of different parts of *Abutilon indicum* (Linn.). World Journal of Pharmacy and Pharmaceutical Sciences. 2006;5(5):1182-96. https://doi.org/10.20959/wjpps20165-6716
24. Ruffell B, Au A, Rugo HS, Esserman LJ, Hwang ES , Coussens LM. Leukocyte composition of human breast cancer. Proceedings of the National Academy of Sciences of the United States of America. 2011;109(8):2796-801. https://doi.org10.1073/pnas.1104303108
25. John C, Jeffrey WP. Macrophages: Obligate Partners for Tumor Cell Migration, Invasion and Metastasis. J Cell. 2006;124:263-66.10.1016.https://doi.org/10.1016/j.cell.2006.01.007
26. Douglas H, Robert W. The Hallmarks of Cancer. Cell. 2000; 57-70.10.1016 /S0092-8674(00)81683. https://doi.org/10.1016/s0092-8674(00)81683-9
27. Noor AL, Elder AS, Ricciardelli C, Oehler MK. Chick chorioallantoic membrane (CAM) assay as an *in vivo* model to study the effect of newly identified molecules on ovarian cancer invasion and metastasis. International Journal of Molecular Sciences. 2012;13(8): 9959-70. https://doi.org/10.3390/ijms13089959
28. Ribatti D, Gualandris A, Bastaki M, Vacca A, Iurlaro M, Roncali L, Presta M. New Model for the Study of Angiogenesis and Antiangiogenesis in the Chick Embryo Chorioallantoic Membrane: The Gelatin Sponge/ Chorioallantoic Membrane Assay. J of Vascular Research. 1997;34:455-63. https://doi.org/10.1159/000159256.
29. Udayashankar AC, Nandhini M, Rajini SB and Prakash HS: Pharmacological significance of medicinal herb *Eclipta alba* L. - A review. Int J Pharm Sci and Res. 2019;10(8):3592-06. https://doi.org/10.13040/IJPSR.0975-8232.10(8).3592-06

30. Udayashankar AC, Rajini SB, Nandhini M, Suhas YS, Niranjana SR, Lund OS, Prakash HS. Acute oral toxicity, dermal irritation and eye irritation study of *Eclipta alba* aqueous extract in Sprague Dawley rats and Newzealand white rabbits. Int Res J Pharm. 2016;103-09. https://doi.org/10.14719/pst.2020.7.4.857

31. Konappa N, Arakere U C, Krishnamurthy S, Gangadharaiah K C, Gubbiveeranna V, Shivaiah N, Chowdappa S, Ramachandrappa N S. Evaluation of *in vitro* antioxidant and antidiabetic activities from *Amomum nilgiricum* leaf extract. Plant Science Today. 2020;7(4):638-44. https://doi.org/10.14719/pst.2020.7.4.857

32. Lakshmi PM, Sarah Jaison S, Muthukumar T, Muthukumar M. Assessment of metal accumulation capacity of *Brachiaria ramosa* collected from cement waste dumping area for the remediation of metal contaminated soil. Ecological Engineering. 2013;60:96-98. https://.org/10.1016/j.ecoleng.2013.07.043

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