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Micropropagation And Antimicrobial Activity Of *Callicarpa Macrophylla* (Priyangu) Against Medically Important Pathogens

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Abstract

Callicarpa macrophylla (Priyangu) a medicinally important plant, represents a class of herbal drug with very strong conceptual and traditional base. In present study extract of leaf, stem showed less antimicrobial activity than seed. They showed antimicrobial activity against three bacterial strains Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas and three fungal Strains Aspergillus fumigatous, Rhizopus oryzae and Aspergillus awamori. Maximum activity was observed in ethanol extract of leaf and stem, Methanol extracts of seed. Phytochemical analysis of the plant extract revealed the presence of phenol, reducing sugar, coumarin and saponins. Micropropogation of C.macrophylla was done using stem as a explant material on MS and Woody media. Which revealed that woody media containing BAP (6-amino benzyl purine) NAA(α-napthaleneacetic acid) gave maximum proliferation response in comparison to MS media. Plant extract (leave, stem and seed) have provide protection against RBC haemolysis and protein denaturation may act as anti-arthritic agent. We propose antiarthritic and antimicrobial activity of C.macrophylla.

Keywords- Micropropagation, antimicrobial activity, haemolysis, phytochemical, Callicarpa macrophylla

Introduction

From the ancient time plants are being utilized as medicines in different ailments (**Balunas and Kinghorn, 2005; Olawuwo et al., 2022**). These medicines we used initially in crude form such as tinctures, teas, poultices, powders, and other herbal formulations (**Samuelsson, 2004; Gonelimali et al., 2018**).

Callicarpa macrophylla Vahl. (Fam-Verbenaceae), commonly known as Priyangu (Gujarat), is one of the erect shrub which is distributed across Asia including India, Nepal, Bhutan, Myanmar, South East Asia, and China **(Billore et al., 2005; Yadav et al., 2012).** *Callicarpa macrophylla* is a species of beautyberry which is a native to the Indian subcontinent. Different diseases can be cured by this plant **(Yadav et al., 2011)**. Bark is used to heal cuts and wounds. Seeds and roots are good for digestion and leaves can be used in rheumatism. In addition, fruits can be used for healing blisters and boils. Extracts of the plant show anti-inflammatory, antifungal and antibacterial activities **(Saifuzzaman Sumon and Paul, 2019; Mishra et al., 2010)**. Stems of the plant has been evaluated for antifungal activity (**Kirtikar et al., 2006)**. The leaves are used in gout and rheumatic pain. Decoctions of the leaves are used in diarrhoea, dysentery and for arresting bleeding. Leaf juice is used in gastric troubles and headache **(Yadav et al., 2011).** The Plant is used in Ayurveda and other folk medicines for the treatment of different diseases and disorder such as tumor, polydipsia, diarrhea, diabetes, dysentery, fever and as blood purifier. Flowers and fruits are useful in rheumatoid arthritis, asthma, catarrh, anorexia, headache, foul ulcers, flatulence, colic diarrhea, dysentery, skin disease, burning sensation, excessive sweating, diabetes, vomiting, fever and general debility **(Sharma et al., 2013).** Root is chewed to relieve rashes on the tongue **(Yadav *et al.*, 2012)**. In the extract carbohydrates, steroids, flavonoids, glycosides, tannins and the inorganic elements like potassium, phosphates, iron and sulphate are reprted in plant extracts. At present, approximately 25% of drugs in modern pharmacopoeia is being derived from plants (phytomedicines) and many others are synthetic analogues built based on prototype of compounds isolated from plants **(Arya et al., 2023)**.

In vitro culture techniques offer a viable tool for mass multiplication of genetically identical plant material and germplasm conservation of rare and endangered medicinal plants **(Shankar et al., 2014; Arya et al., 2023)**. **Murch *et al.*, (2003)** considered in vitro propagation of medicinal plants a successful strategy that addresses the problems associated with supply and variability in the product quality. In view of increasing global popularity of herbal medicines, their cultivation would ensure constant supply and is also an important alternate source of income. **Chung *et al.*,** (**2004)** evaluated antimicrobial activities of plant extracts of *Callicarpa erioclona Schau*. (Verbenaceae), *Callicarpa farinosa Roxb*. (Verbenaceae), *Sphonodesma friflora Wright* (Verbenaceae), and *Homalium panayanum* F. Villar (Flacourticeae) against Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria and fungi *Candida albicans*. These plant extracts exhibited strong antimicrobial activities against methicillin-resistant *Staphylococcus aureus* strains (MRSA) **(Paprikar, and Paprikar, 20121)**. Phytochemical screening of the members of the genus *Callicarpa*, revealed the presence of flavonoids, essential oils, and terpenoids **(Shankar et al., 2014; Soni et al., 2014)**. Evolution of antimicrobial and anti fungal activity of *Callicarpa arborea* extract showed 24-28 mm and 29-34 mm zone of inhibition against fungi and bacteria respectively (**Umachandur *et al.*, 2015**). Micropropagation has many advantages over conventional propagation of plants like regeneration, transformation and cryopreservation **(Channuntapipat *et al.*, 2000; Jayaraman and Variyar, 2015)**.

Materials and Methods

Sample collection

Plant material was collected from Forest Research Centre (FRC) Haldwani. Chemicals used in this study were of analytical grade.

Sample preparation for antimicrobial assay

Antibacterial and antifungal activities were tested in nutrient agar according to **Deattu *et al.*, (2012)** and on Sabouraud dextrose medium.

Plant samples were washed thoroughly and dipped in 70% ethanol for sterilization. The dried samples were then chopped in fine pieces and shade-dried at ambient temperature (31 ºC). Plant material was powdered using an electronic blender. The powdered mixture was then soaked in three different solvents (petroleum ether, ethanol and methanol) for 72 hrs. After filtration the contents using Whattmann No. 1 filter paper, filtrate was left at room temperature for 48 hrs to evaporate partially. The remaining crude extracts thus obtained were used for further analysis.

The test microorganisms used for antimicrobial test were *Pseudomonas species, E. coli, B. subtilis, S. aureus.* To test antifungal activity, fungal strains of *R. oryzae, A. awamori, T. viride, C. oryzae* and *A. fumigatous* were used. The bacterial and fungal strains were maintained in the Microbiology laboratory of the Department for future use.

Antimicrobial activity Assay

The antimicrobial activity of the plant extracts (leave, stem, and seed) in Petroleum ether, methanol and ethanol was evaluated by disc diffusion method **(Arya et al., 2023).**

Whatmann No. 1 filter paper discs (1.0 cm dia.) soaked in three different solvents were placed carefully in the centre of Petri-plates containing the solidified media. The medium was inoculated with 1ml of active bacterial culture. The results of antimicrobial activity were compared with the control containing same concentration of same solvents. The plates were incubated at 37º C for 24 hrs for bacterial culture and for fungal culture the plates were incubated at 28 ºC for 48 hrs. The Antimicrobial Activity of the above mentioned different solvent extracts of *Callicarpa* spp. *(*stems, leaves, seed) were assayed by measuring the resultant Zones Of Inhibition (ZOI) by the help of ruler.

Preliminary phytochemical analysis-

The extracts obtained were subjected to qualitative test for identification of chemical constituents of the selected parts of the plant **(Olawuwo et al., 2022; Arya et al., 2023).** Crude extracts for antimicrobial activity were used for preliminary phytochemical analysis for steroids, reducing sugars, phenols, coumarin and saponins.

Micropropagation of Callicarpa macrophylla

Murashige and Skoog (1962) and Woody plant medium **(Lloyd G and McCown B; 1980)** medium was used as a basal medium. It was supplemented with growth regulators, vitamins as per requirement of individual experiments. Growth media for micropropagation was prepared by

Stock solutions of growth regulators (BAP, NAA, Zeatin, IAA) were prepared at a concentration of 25 mg/50ml into 1 N NaOH solvent. The basal medium was supplemented with various concentrations of different plant growth regulators, which were stored as stock solutions (Table -1).

Preparation of the medium for micropropagation-

Stock solutions of micro and macronutrients, vitamins and growth regulators were mixed stepwise according to their relative concentration in one litre conical flask. The required amount of sucrose, NH4NO3 and KNO3 were added freshly to the medium. The medium was stirred thoroughly and its ph was adjusted to 5.8 with 0.1N NaOH or 0.1 N HCL. The required amount of agar i.e. 8 gram was added to the medium and diluted to the final volume of one liter. The medium was therefore boiled till it became a transparent solution. It was poured into glass bottles / conical flasks and thereafter, it was capped /cotton plugged and autoclaved.

Surface sterilization of explants-

Stem of the *Callicarpa macrophylla* wash with tap water (dip for 20 minutes). Wash with Tween -20 for 30 minutes. Then wash with double distilled water for 5 minutes. Wash with 70% alcohol for 1 minute. Treat with 0.1% hgcl2 for 5 minutes. By 2 % sodium hypochlorite treat it for 2 minutes. At last wash with double distilled water for 3 minutes.

Combination of growth regulators tested for establishment/Shoot proliferation from nodal explant -

The inoculation culture bottles/flasks were kept in the culture room maintained at a temperature 27 ± 1^0C and a high light intensity. A photoperiod of 16 hours light and 8 hours dark was maintained in the culture room. Sub culturing of explants was done after an interval of three to four weeks **(Table 1)**.

Table 1: Growth regulators tested for establishment/Shoot proliferation from nodal explant

S. No.	Media	BAP(mg/l)	NAA(mg/l)	Zeatin(mg/l)	IAA(mg/l)
1	MS	1	0.5	-	-
2	MS	-	-	1	0.25
3	**WP1**(WM **)**	1	0.5	-	-
4	**WP2**(WM)	-	-	1	0.25
5	**WP3 (**WM+PVP(50mg/l))	1	0.5	-	-
6	**WP4 (**WM+PVP(50mg/l))	1	-	-	0.25

In vitro* Anti-arthritic activity of ethanolic extract of *Callicarpa macrophylla

Preparation of Ethanolic Extract

Sample of (leaves, stem and seeds 500gm) of *C. macrophylla* were ground individually into moderately coarse powder & extracted with solvent ethanol for 27 hrs. The solvent was removed under reduced pressure. The ethanolic extract was used for the preliminary anti-arthritic activity.

Inhibition of albumin denaturation-

The reaction mixture (0.5ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of *C. macrophylla* extracts at different concentrations. The samples were incubated at 37^0 C for 30 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured using spectrometric methods at 660 nm for control test 0.05 ml distilled water was used instead of extracts while product control test lacked bovine serum albumin. Diclofenac sodium (standard drug) was used as reference drug and treated as such for determination of absorbance. The percentage inhibition of protein denaturation was calculated as follows:

% of inhibition = 100× [Vt / Vc – 1]

Where, Vt = absorbance of test sample, Vc = absorbance of control.

Membrane stabilization test

Preparation of Red Blood cells (RBCs) suspension-

Fresh whole human blood (10 ml) was collected and transferred to the heparin zed centrifuged tubes. The tubes were centrifuged at 3000 rpm for 10 minutes then washed three times with equal volumes of normal saline. The volume of the blood was measured and reconstituted as 10 % v/v suspension with normal saline.

Heat Induced Hemolysis ethanolic extracts of leaves and stem-

Firstly, the reaction mixture were prepared (2 ml) consisted of 1 ml of 10 % RBC suspension and 1 ml of varying concentration of extract (ethanolic extract of leaves, stem, seed) so that final the concentrations were: 50,100, 200, 400, 800 µg / ml. All the centrifuge tubes containing reaction mixture were incubated in a water bath at 56 ^{0}C for 30 minutes. At the end of incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 minutes and the absorbance of the supernatant was taken at 560 nm. The reaction mixture (2 ml) consisted of 1 ml of 10 % RBC suspension and 1 ml of saline was serve as control test tube (**Kumari *et al.*, 2015)**. Asprin was used as reference drug at the concentrations of 50, 100, 200, 400, 800 µg / ml. It was treated similarly as reaction mixture for the determination of absorbance. The percentage of protein-membrane stabilization activity was calculated by using the following formula:

% of inhibition = 100× [Vt / Vc – 1]

Where, Vt = absorbance of test sample, Vc = absorbance of control.

Results and Discussion

The present investigation entitled "Micropropagation, Anti-arthritic activity, Phytochemical Analysis and Antimicrobial Activity of *Callicarpa macrophylla*" was under taken with the view to collect precise information on various aspects related to diversity and its medicinal value **(Arya et al., 2023)**. Preliminary phytochemical analysis and antimicrobial activity materials were soaked in three different solvents (Methanol, Ethanol, Petroleum Ether). Colour changes were recorded. The phytochemical test of the leaf crude extracts showed that *Callicarpa macrophylla* contains various phytochemical constituents such as reducing sugar, phenol, saponins, and coumarin. The stem contains three phytochemical constituents phenol, saponins and coumarin but reducing sugar were absent. The seed contains only coumarin but no reducing sugar, phenol and saponins **(Table 2).** Our results are similar to **Ping *et al.*, (2006),** phytochemical screening of several members of the genus *Callicarpa*, revealed the presence of flavonoids, essential oils, and terpenoids **(Yadav et al., 2011,2012)**.

Table 2: Preliminary phytochemical analysis of *Callicarpa macrophylla* of leaf, stem, and seed extract

Presence/absence of bioactive components	Petroleum ether			Ethanol			Methanol		
	Leaf	Stem	Seed	Leaf	Stem	Seed	Leaf	Stem	Seed
Anthraquinones	-	-	-	+	+	-	+	+	-
Catechin	+	+	-	+	+	-	+	+	-
Coumarin	+	+	+	+	+	+	+	+	+
Flavonoids	-	-	-	-	-	-	+	+	-
Phenols	+	+	-	+	+	-	+	+	-
Quinones	+	+	-	+	+	-	+	+	-
Saponins	+	+	-	+	+	-	+	+	-
Steroids	+	+	-	+	+	+	+	+	-
Tannins	+	-	-	+	-	-	+	-	-
Terpenoids	+	-	-	+	-	-	+	-	-
Reducing Sugar	+	-	-	+	-	-	+	-	-

Against *S. aureus* the maximum zone of inhibition was recorded in the case of methanolic crude extract of leaves (27mm). For *B. subtilis* the maximum zone of inhibition was recorded in case of petroleum ether (13mm) crude extract of leave while methanol crude extract did not show any activity. In case of *Pseudomonas* all the three extract showed activity. Maximum activity was recorded by petroleum ether crude extract of leaves (10mm). For *E. coli* methanolic (14mm), petroleum ether crude extracts of leaves (10mm) showed activity.

Antibacterial activity of stem of *Callicarpa macrophylla*-

Crude plant extracts using ethanol, methanol & petroleum ether solvents showed activity against *S aureus*, with the maximum activity recorded in methanol (22mm). *E. coli* all three crude extract showed activity but the maximum zone of inhibition was recorded in case of petroleum ether (14mm). *B. subtilis* petroleum ether (18mm) showed activity. Ethanol and methanol crude extract did not show any activity. Methanol extract showed maximum activity against *Pseudomonas* (12mm), other than ethanol and petroleum ether crude extract.

Antibacterial activity of seed of *Callicarpa macrophylla*-

Against *S.aureus* only methanol(9mm), petroleum ether (12mm) crude extracts showed activity. While ethanol crude extract did not show activity. For *B. subtilis* all three crude solvents did not show any activity. In case of *Pseudomonas* methanolic (15mm) crude extract showed high activity while petroleum ether crude extract gave no activity. While in case of *E. coli* except ethanol all crude extracts showed activity. Maximum activity showed by methanol (22mm) than petroleum ether (12mm) **(Fig 1)**.

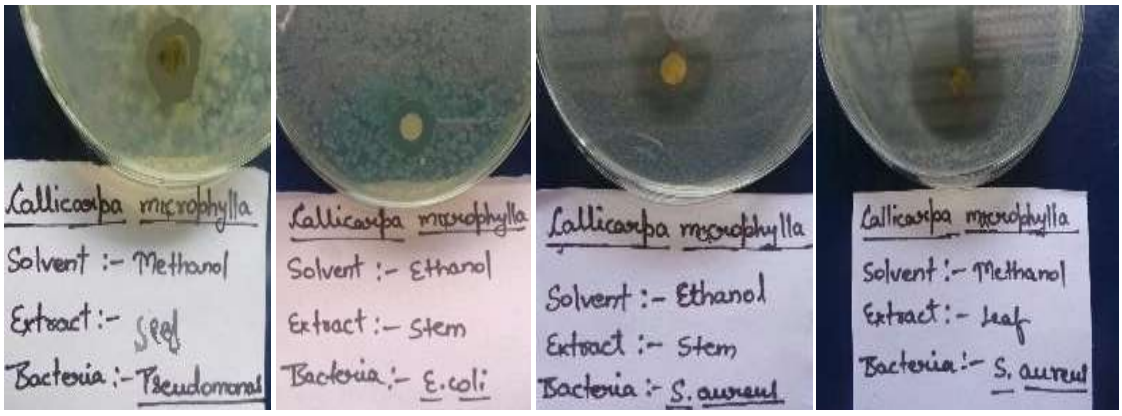

Figure 1: Zone of inhibition of *Callicarpa macrophylla* plant extracts (stem, seed, leaf) against different bacteria in different solvent containing nutrient agar medium

The above graph **(Fig 2)** revealed that out of various extracts, the leaves crude extract was more effective against the bacterial strain in comparison to stem and seed crude extract. Further analysis showed that from

various crude extracts of leaf, petroleum ether & methanolic crude extracts were more effective followed by ethanolic. In case of stem petroleum ether crude extract was more sufficient to inhibit the bacterial growth followed by methanol and ethanol. The findings showed that the leaves of *C. macrophylla* is an interesting source of biologically active compounds that may be applied for prophylaxis and therapy in human which justifies their traditional use to treat infectious diseases and hence reinforce the importance of the ethno botanical approach as a potential source of bioactive substances **(Gonelimali et al., 2018)**. **Chung *et al*.,(2004)** evaluated the antimicrobial activities of plant extracts of *Callicarpa erioclona Schau*. (Verbenaceae), *Callicarpa farinosa Roxb*. (Verbenaceae), *Sphonodesma friflora Wright* (Verbenaceae), and *Homalium panayanum F. Villar* (Flacourticeae) against Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria using agar dilution method and showed 15mm to 22 mm zone of inhibition.

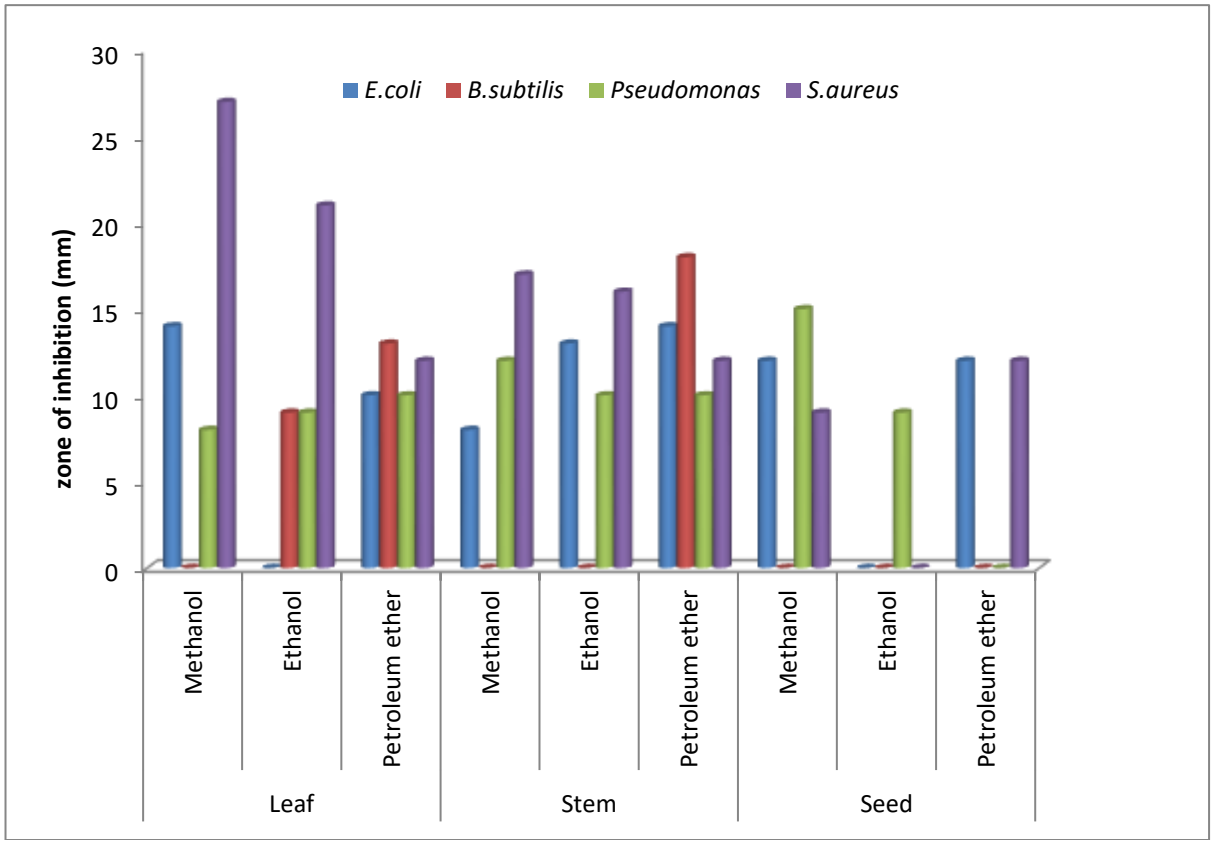

Figure 2: Graph showing comparison of leaf, stem and seed crude extracts against different bacterial strains with different solvent.

Antifungal activity of leaves of *Callicarpa macrophylla*

Against *A.fumigatous* all crude extract methanol(8mm), ethanol(12mm) and petroleum(9mm) ether shows activity**.** For *R.oryzae* crude extract Methanol(10mm) & ethanol(10mm) extract shows activity while Petroleum Ether did not show any activity**.** In case of *A.awamori* Ethanol (10mm) and petroleum ether (9mm) shows activity while Methanol did not show any activity. While in case of *Tricoderma viridae* and *curvularia oryzae* all three Methanol, ethanol and petroleum ether did not show any activity **(Fig 3).**

Antifungal activity of stem of *Callicarpa macrophylla*

Against *A. fumigatous* only petroleum ether (9 mm) crude extract shows activity while the Methanol and ethanol extract did not show any activity. For *Rhizophous oryzae* the maximum zone of inhibition was recorded in methanol (12mm), ethanol (12mm) crude extract while petroleum ether (9mm) show significant activity. In case of *A. awamori*, *T.viridae and C.oryzae* all three Methanol, ethanol and petroleum ether shows no activity **(Fig 3).**

Figure 3: Zone of inhibition of *Callicarpa macrophylla* plant extracts (stem, seed, leaf) against different fungi in different solvent containing medium.

Antifungal activity of seed of *Callicarpa macrophylla*

Against *A. fumigatous* all three crude extract ethanolic (12mm), methanolic (9mm), petroleum ether (9mm) shows activity. For Rhizopus oryzae only ethanol (12mm) and methanol (9mm) crude extract shows activity while petroleum ether did not show activity. In case of *A. awamori* two crude extract ethanol (12mm) and petroleum ether (9mm) shows activity while methanol did not show activity. While in case of *T.viridae and C.oryzae* all three methanol, ethanol and petroleum ether shows no activity **(Fig 3).**

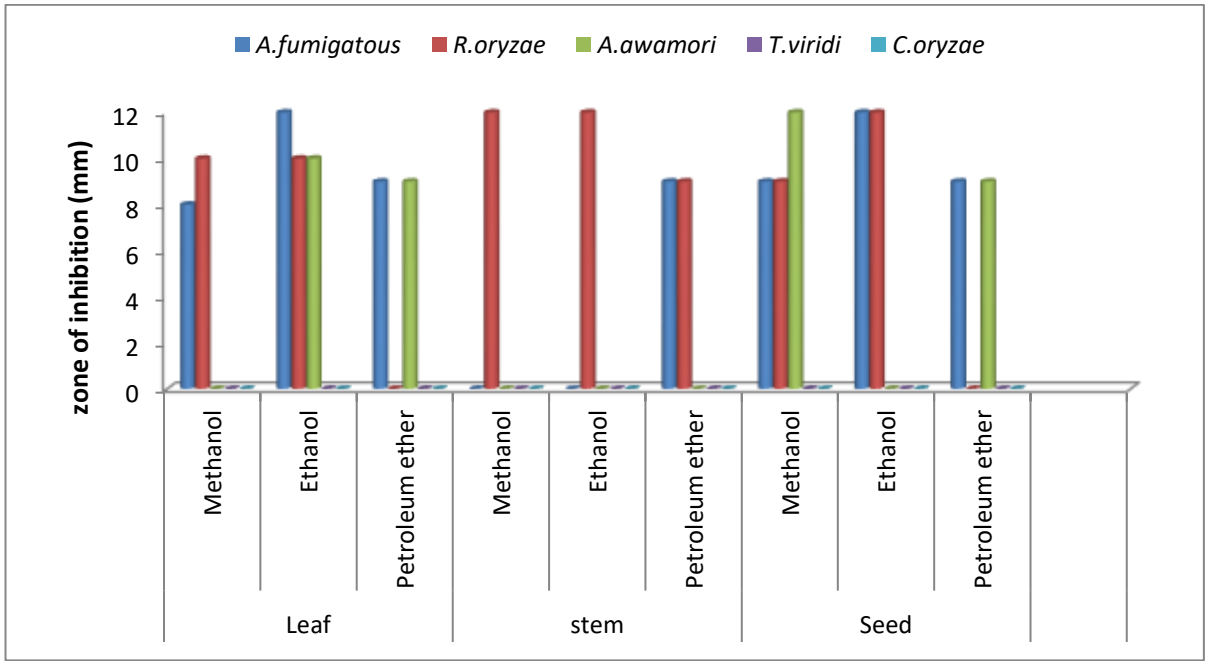

Figure 4: Graph showing comparison of leaf, stem, and seed crude extracts against different fungal strains with different solvent.

The result from the above graph **(Fig 4)** showed that among various crude extracts of leaf, stem and seed extract effectively inhibited the growth of various fungal strains. From various extracts of leaf, ethanol was more effective followed by methanol and petroleum ether. In case of stem petroleum ether crude extract gave maximum activity while methanol and ethanol gave minimum activity against the fungal strain. In case of seed, methanol gave maximum activity in comparison to ethanol and petroleum ether. Same findings were obtained by **Yadav *et al.*, (2012)** evaluated antifungal activity of ethanolic and aqueous extract of stem of Callicarpa macrophylla against *G. fujikoroi*, *C. neoformans*, *C. albicans*, *M. verrucaria*, *A. niger*, *N. crassa* and *R. oligosporus*. They found that the ethanolic extract of stem exhibited antifungal activity of all that strains. Largest zone of inhibition was recorded against *G.fujikoroi*, but aqueous extract doesnot exhibited antifungal activity.

Micropropagation

For shoot proliferation three media were tested (MP1 MP2 & MP3) out of which all three media gave no response. It was found that in media MP1 supplemented with MS+(α-napthaleneacetic acid (NAA 0.5mg/l) +BA (benzyl adenine), MP2 supplemented with MS+Zeatin(1mg/l)+IAA(0.25mg/l) and MP3 supplemented

with MS+NAA(0.5mg/l) +BAP(1mg/l) gave no efficient results. It has been found that out of 5 explant, no one plant regenerate in these medium.
But shoot proliferation in case of woody media, four media were tested (WP1, WP2, WP3, WP4) out of which WP3 gave good response in comparison to WP1, WP2 and WP4 . It was found that in media WP1 supplemented with BA, only increase in number of leaves was observed. While when amount of NAA and BA (WP3) increase was added in the media it increased the number of leaves along with their enlargement and also it increases the number of shoots. So we use woody media for proliferation **(Fig 5)**.

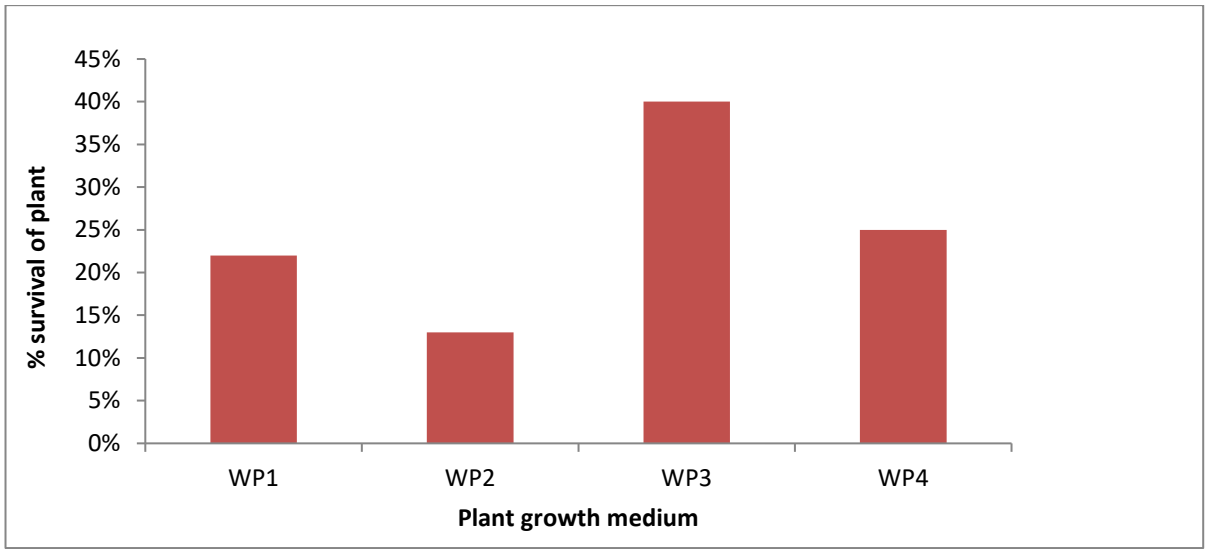

Figure 5: Percent survival of plants in different composition of plant growth medium

Shoot Proliferation in Different Compositions of Media
While study of *in vitro* propagation of *Callicarpa macrophylla* using nodal explant it was found that MS media supplemented with BA, NAA & PVP not gave maximum proliferation response. It shows that WPM gave good response in comparison to MS media so it was concluded that basal salt compostion also plays important role in stabilishment/shoot proliferation of explants.
According to **Monney *et al.*, (2016)** Cryptolepis sanguinolenta (medicinal plant species), for shoot induction, MS (Murashige and Skoog) nutrient medium supplemented with MS vitamins, 30 g/L sucrose, 3% gelrite and various auxin and cytokinin combinations. Treatments involved 6-benzyladenine (BA) at 0.5, 1.0, 1.5, 2.0, 2.5 or 3.0 mg/L in combination with 0.1 mg/L Indole 3-butyric acid (IBA) or Naphthaleneacetic acid (NAA). BA and IBA combinations were found to be more efficient in shoot regeneration than the BA and NAA combinations.

In vitro Anti-Arthritic activity of *Callicarpa macrophylla*-
Anti-arthritic effect of *Callicarpa macrophylla* was studied significantly by testing various *invitro* parameters. For that purpose various extracts were made with ethanol **(Singh *et al.*, 2011)**.

Inhibition of protein denaturation of ethanolic extract of *Callicarpa macrophylla*
In the present study it was observed that *C. macrophylla* at different doses levels (50,100,200,400,800µg/ml) provided significant protection against denaturation of proteins. The effect of *C.macrophylla* (leaves,stem and seed) on inhibition of protein denaturation is shown in **(Table 3)**.

Table 3: Inhibition of protein denaturation of ethanolic extract of *Callicarpa macrophylla*

Test Sample	Conc.(µg/ml)	% protection			
		Leaves	Stems	Seeds	Diclofenac sodium (Std. drug)
Ethanolic extract of *Callicarpa macrophylla*	50	12.23	10.03	12.23	96.8
	100	14.99	11.47	13.53	131.66
	200	17.27	14.24	15.27	151.82
	400	20.03	16.33	19.23	182.93
	800	22.80	19.29	24.46	210.6

Invitro Anti-Arthritic activity by inhibition of protein denaturation method-
In case of seed extract maximum percentage of inhibition was found at the concentration of 800µg/ml which shows 24.46% inhibition followed by 400 & 200µg/ml conc. While the standard diclofenac inhibits 96.8% at 50µg/ml. For the leaves ethanolic extract 800µg/ml shows 22.80% inhibition followed by 400µg/ml (20.03% inhibition). For the stems ethanolic extract 800µg/ml shows 19.29% inhibition followed by 400µg/ml (16.33% inhibition) **(Fig 6)**. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonds. From the result of the present study it can be stated that leaves, stems & seeds extracts are capable of controlling the production of auto antigens due to invitro denaturation of protein in rheumatic disease. The anti-inflammatory activity of ethanolic leaves, stems and seeds extracts was assessed by *invitro* HRBC membrane stabilization method **(Singh *et al.*, 2011)**. The extracts concentration ranges from 50,100,200,400,800 µg/ml, protects human erythrocytes membrane against lysis induced by hypotonic solution.

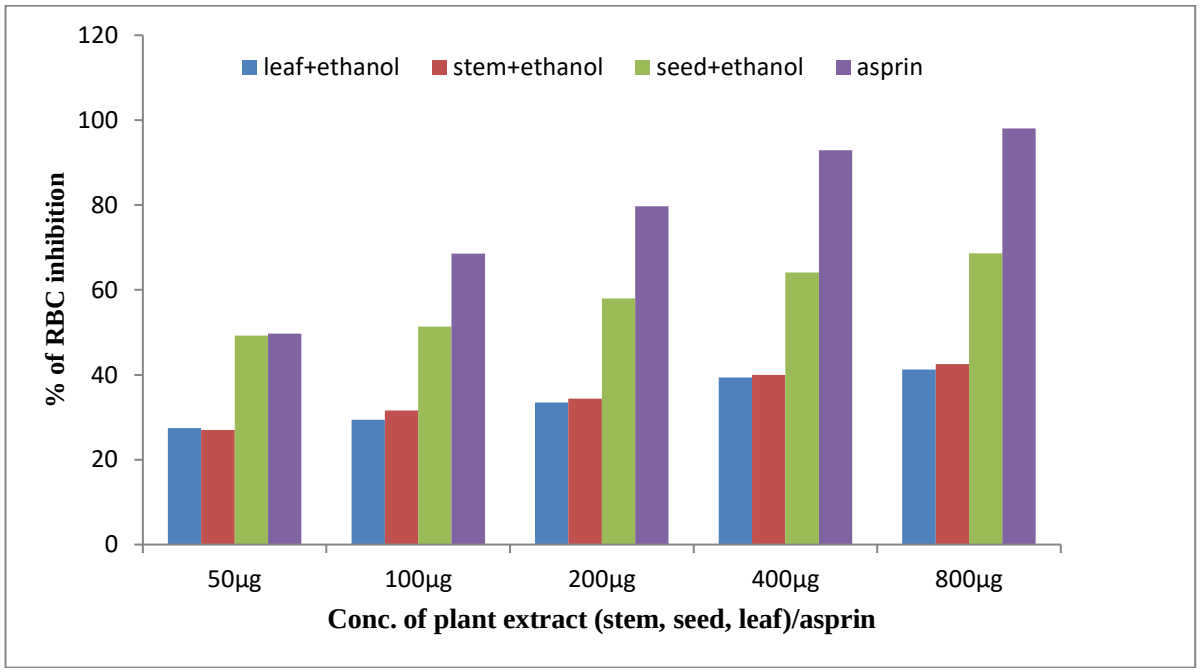

Figure 6: This graph shows anti-inflammatory activity by Membrane Stabilization

For Ethanolic leaves extract-conc. of 800µg/ml inhibits 41.27% of RBC, while 400µg/ml inhibited 39.33% of RBC, while asprin inhibits 49.7% of RBC at the conc. of 50µg/ml. For Ethanolic stems extract-conc. of 800µg/ml inhibits 42.53% of RBC, while 400µg/ml inhibited 39.94% of RBC(Table-9), but asprin inhibits 49.7% of RBC at the conc.of 50µg/ml(Table-10). For Ethanolic seeds extract-conc. of 800µg/ml inhibits 68.63% of RBC, while 400µg/ml inhibited 64.08% of RBC, while asprin inhibits 49.7% of RBC at the concentration of 50µg/ml(Table-10).

Since human red blood cell membrane are similar to lysosomal membrane component, the prevention of hypotonicity induced HRBC membrane lysis was taken as a measure of anti-inflammatory activity of drug.The result obtain demonstrated that of leaves, stems and seeds of *Callicarpa macrophylla* can significantly and dose dependently inhibits HRBC haemolysis. From the study of this experiment it may be concluded that ethanolic seeds has good membrane stability. So it can be used as potent anti-arthritic agent for the treatment of arthritics.

According to **Kumari *et al.*, 2015** Protein denaturation is well documented method for this analysis and membrane stabilization also reflects the effect of extracts on cellular membrane like red blood cells. HRBC membrane are similar to lysomal membrane components. The prevention of hypotoxicity induce HRBC membrane lysis is taken as a measure of anti inflammatory activity of drugs. The methanolic extract of *Rhizophora mucronata* source significant antiinflammatory activity at the concentration of 500 mg which is comparable to the standard drug diclofenac sodium.

Conclusion

The preliminary Phytochemical analysis showed that in various crude extract of leaf reducing sugar, phenol, coumarin & saponins phytochemical constituents were present. The preliminary Phytochemical analysis showed that various crude extract of stem contain three phytochemical constituent phenol, saponins, coumarin except reducing sugar. The preliminary Phytochemical analysis showed that in various crude extract of seed had only coumarin while reducing sugar, phenol, and saponin are absent. Antimicrobial activities of various crude extract were compared with same concentration of control (same solvent). Screening of antibacterial activity of leaf showed that it is potent antibacterial agent against *S. aureus, B. subtilis, Pseudomonas* and *E. coli* petroleum ether crude extract showed maximum activity followed by methanol crude extract. While ethanol show least activity. Study of antibacterial activity of stem showed that it is potent antibacterial agent against *S. aureus, B. subtillis, Pseudomonas* & *E. coli*. Maximum activity was shown by petroleum ether crude extract followed by ethanol while methanol crude extracts were unable to inhibit the growth of *B. subtilis.* In case of antibacterial activity of seed it was found that methanol crude extract is able to inhibit *Pseudomonas, S. aureus , E. coli* while ethanol crude extract was found effective against *pseudomonas* only. In case of antifungal activity of leaf, maximum activity was found against *A. fumigatous* Further investigation revealed that ethanol crude extract was the potent antifungal agent followed by petroleum ether and methanol. Antifungal activity of stem revealed that petroleum ether crude extracts were able to inhibit *A. fumigatous, R. oryzae, A. awamori*. followed by ethanol extracts while methanol crude showed less activity against the fungal strains. Antifungal activity of seed revealed that methanol crude extract give best antifungal activity than ethanol and petroleum ether. Cultivation of valuable medicinal plants is one of the best techniques for its protection. We can also use classical methods like cutting, bulbs. Nowadays micropropagation is very popular method which is used for commercial, economical, rapid propagation and *exsitu* conservation of rare, endemic and endangered medicinal plants.

While studying *in vitro* propagation of *C. macrophylla* using nodal explant it was found that woody media supplemented with BA, NAA and PVP enhances shoot proliferation and elongation in comparison to media supplemented with IAA and BA alone. The inhibition of protein denaturation and membrane stabilization was studied to establish the mechanism of anti-arthritic activity of *C. macrophylla*. Therefore, our *in vitro* studies on extract of *C. macrophylla* demonstrate the significant anti-arthritic activity. Hence, this mangrove plant can be used as a potent natural anti arthritic agents. The results show that the extracts of *C. macrophylla* exhibited anti-arthritic activities might be due to the presence of active principles such as polyphenolic content, triterpenoids, alkaloids and flavanoids. From the results of the study, it can be concluded extract of *C. macrophylla* possessed antiarthritic property.

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