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PHYTOPHARMACONOSTICAL EVALUTION OF INDIAN MEDICINAL PLANTS ACORUS CALAMUS AND GYMNEMA SYLVESTRE

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ABSTRACT

Medicinal plants show different kinds of medicinal activities that are being used in India and China among these, rhizome of Acorus and leaves of Gymnema were used in our study for the investigation of various medicinal properties like wound healing, anti-oxidant, anti-inflammatory and anti-bacterial were investigated using various assays from the extractions of solvents like hexane, ethyl acetate (EA) and methanol. The mentioned medicinal properties were investigated by performing assays like scratch assay, 2, 2-diphenyl-1-picrylhydrazyl assay, Hydrogen peroxide assay, Human Red Blood Cell assay and well diffusion method. Our research study showed that Acorus calamus as well Gymnema sylvestre possess all the investigated medicinal activity at various concentrations at various solvents.

KEYWORDS: Acorus calamus, Gymnema sylvestre, scratch assay, well diffusion method, 2, 2-diphenyl-1-picrylhydrazyl.

INTRODUCTION

Plants used to excrete some ergastic substance which seems to be medicine for humans. These ergastic substances are being deriving the attention of many scientists in phyto-pharmacology. Ergastic substances were also known as secondary metabolites or phytochemicals; isolation and identification of these secondary metabolites leads to the

discovery of many medicines for a number of diseases for certain decades. This derived my attention towards two different Indian medicinal plants Acorus calamus and Gymnema sylvestre to investigate some of the medicinal properties like wound healing, anti-inflammatory, anti-oxidant and antibacterial. Acorus calamus: It belongs to Acoraceae family, whose native is Europe, Russia, japan, India and china. It is well grown in swampy regions. It is also known as "baby medicine", since it cures digestive disabilities, flatulence, anorexia, dysentery etc.

Gymnema sylvestre: It has also being used as anti-diabetic medicine in India and Sri Lanka. To this, it treats weight loss, cardiac problems, constipation, liver diseases, rheumatoid arthritis and gout; reduce blood cholesterol and triglyceride level. It also possesses various medicinal properties like Anti-Inflammatory[1] anti-diabetic, anti-microbial[2], anti-viral, anti-venom[3] and anti-oxidant

AIM OF STUDY

To detect the presence of wound healing, anti-inflammatory, anti-bacterial and antioxidant activity in the rhizome extracts of Acorus calamus and leaves extract of Gymnema sylvestre.

MATERIALS AND METHODS

Plant Collection

Powder form (100g) of rhizomes of Acorus Calamus and leaves of Gymnema Sylvestre were collected from country medicine shop in Saidapet, Chennai, TamilNadu.

Plant Extraction

Powdered leaves and rhizomes of Gymnema sylvestre and Acorus calamus respectively were sequentially extracted (48hrs) with hexane, ethyl acetate (E.A), Methanol respectively. The extracts were made to evaporate under room temperature. The evaporated extracts of A.calamus and G.sylvestre were subjected to various assays like DPPH, H_2O_2, HRBC, scratch assay and well diffusion method.

Preparation of Stock Solution of Sample

10mg/ml of stock has been prepared with each extracts of G.sylvestre and A.calamus in methanol separately. This was used for all assays.

Scratch Wound Healing Assay

3T3L1 cells were cultured with appropriate culture medium by incubating for 24 hours in 24 well plates (70-80% confluence), to which a scratch is made with sterile 1ml micropipette tip. Scratched cells were washed twice with the medium and supplemented with fresh medium along with Ethyl acetate extract of A.calamus and methanol extracts of G. Sylvestre (100,200µL). After 48 hours of incubation, the cell proliferation at the site of scratch has been viewed under microscope by fixing the cells for 30 min with 3.7% paraformaldehyde, which was then stained with crystal violet (1%) in ethanol (2%)[4]

The images of cell proliferation at the site of scratch have been taken. (Image 1& 2)

ANTI-INFLAMMATORY ACTIVITY

HRBC suspension

Blood is collected from a volunteer individual (5mL) to which EDTA is added and centrifuged for 5min at 3000 rpm, whose supernatant is discarded and pellet is suspended with PBS (10%).

Hypotonicity induced haemolysis (HRBC) assay

Ethyl acetate, methanol extracts of G.Sylvestre (fig.3) and hexane, ethyl acetate, methanol extracts of A.Calamus (fig.4) (200,300,500µg) were make up to 1ml with PBS. To which hypo saline (2ml) and HRBC suspension (0.5ml) were incubated at 37˚C for about 30 min. which was then centrifuged at 3000 rpm, whose supernatant is decanted and haemoglobin content was estimated at 560 nm.

ANTI-OXIDANT ACTIVITY

Standard stock solution of ascorbic acid for DPPH assay and H_2O_2 assay

A stock solution of about 10mg/ml of ascorbic acid is prepared with methanol, whose spectral readings of various concentration (200, 400, 600, 800, 1000 µg) were plotted with graph.

DPPH assay

Ethyl acetate, methanol extracts of leaves of G.sylvestre (Fig 5) and hexane, ethyl acetate and methanol extract of rhizomes of A.calamus (Fig 6) (200, 400, 600, 800, 1000µg) from stock solution 10mg/ml were make up to 1.5ml by addition of methanol. DPPH solution (1.5 ml) is added to this, whose O.D values were taken at 517nm after incubation of 30 min in dark.[5]

H_2O_2 assay

40mM of H_2O_2 solution in phosphate buffer (0.6ml) is added to all the 3, rhizome and leave extracts of A.Calamus and G.Sylvestre (200-1000µg). After 10min of incubation, the extracts were made up to 1.5ml by addition of PBS accordingly, and then whose spectral values at 230nm were taken and plotted in graph. (Fig: 7&8)

ANTI-BACTERIAL ACTIVITY

Micro-organism

Leave extract of G.sylvestre were subjected to test anti-bacterial activity in *Lactobacillus, Streptococcus Mutans* (gram +ve), and *Enterococcus Faecalis, Shigella* (gram –ve).

Whereas, rhizome extract of A.calamus were tested with *Lactobacillus, Streptococcus Mutans* (gram +ve) and *Klebsilla, Pseudomonas Aeruginosa* (gram –ve) for its anti-bacterial activity using well diffusion method.

Well diffusion method

LB-agar medium is prepared which is autoclaved (121˚C at 15 for 15 min) and plated. In which the mentioned bacterial culture were streaked in which using sterile micro tip wells were punched (5 wells per plate). To these wells, hexane, ethyl acetate (E.A), methanol extracts of A.calamus and G.sylvestre (200,400,600,800 µg) were loaded to which Tetracycline is used as control in all plates. After incubation for 48 hours, zone of inhibition is measured.[6] (Table 1& 2).

RESULT

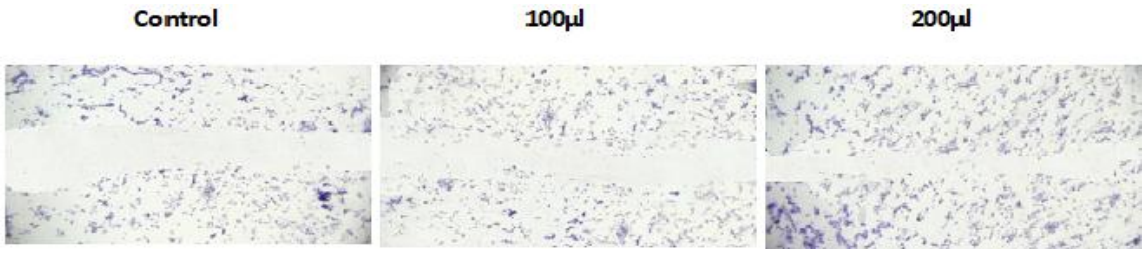


Fig: 1 Scratch assay of Ethyl acetate leaves extract of G.Sylvestre.

At 200µl concentration of ethyl acetate leaves extract the cells showed highest proliferation rate in healing the wound (at the site of scratch) than at 100µl concentration.

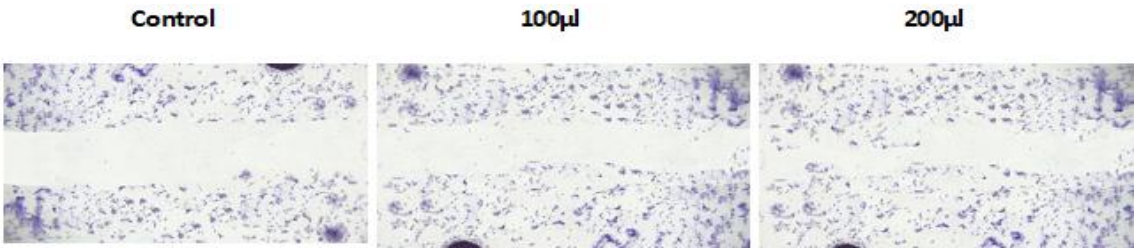


Fig: 2 Scratch assay for methanol rhizome extract of A.calamus.

The rate of cell proliferation at the site of scratch (say wound) was high in 200µl con of rhizome methanol extract of A.calamus when compared to 100µl con.

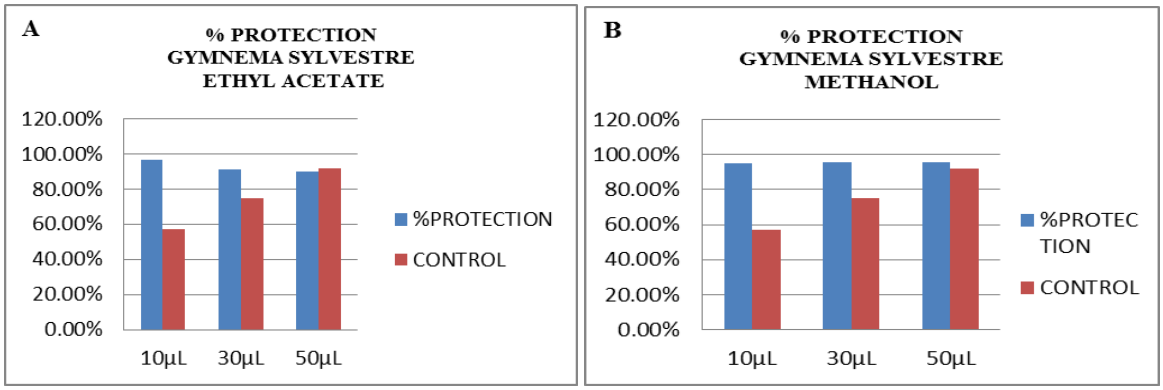
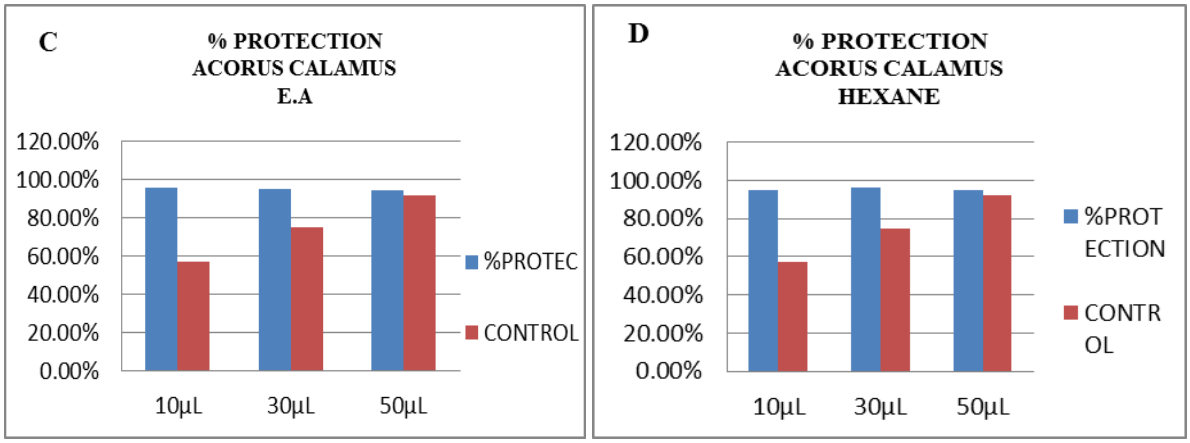


Fig3: Graph A&B represents the anti-inflammatory activity by HRBC assay of Ethyl acetate and methanol extract of Gymnema sylvestre.

Spectral values of ethyl acetate (EA) and methanol extract of Gymnema sylvestre were compared with the positive control OD values, which showed highest anti-inflammatory activity at all concentrations (10,30,50µl) than positive control values.



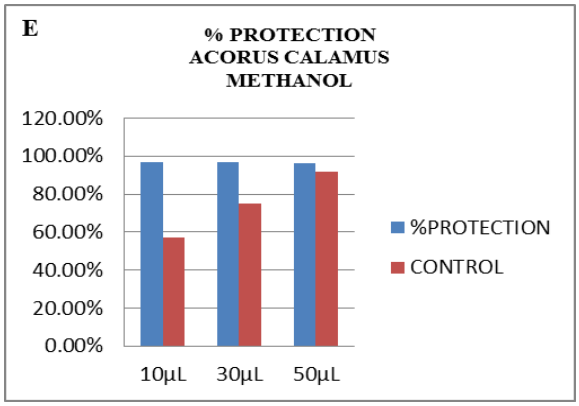


Fig4: Graphical representation of HRBC assay of ethyl acetate, hexane and methanol extracts of A.calamus.

C, D, E graphs shows the %protection of haemolysis by ethyl acetate, hexane and methanol extract of A.calamus respectively. In which, at all concentrations (10, 30, 50µl) of extract, the anti-inflammatory activity is at same concentration.

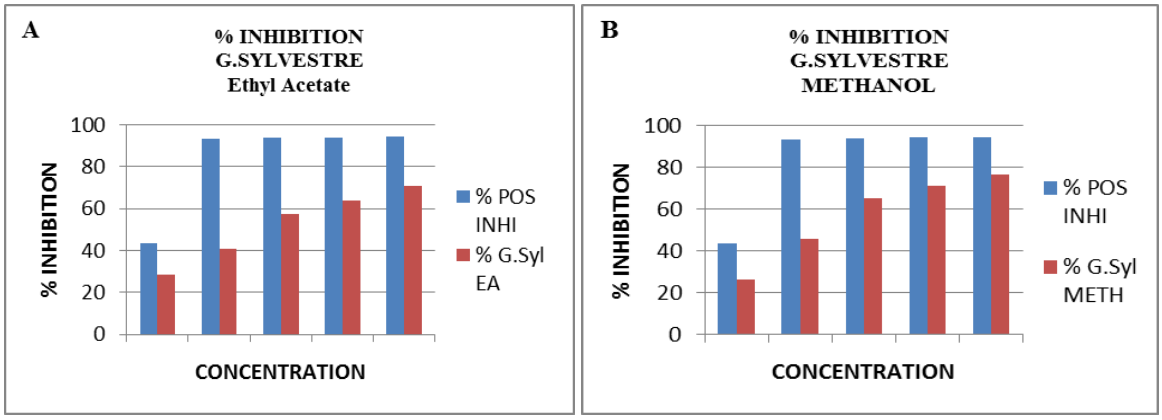


Fig5: A and B graph represent the DPPH assay to predict anti-oxidant activity of Ethyl acetate and Methanol extracts of G.sylvestre.

Ethyl acetate and methanol extracts of G.sylvestre possess anti-oxidant activity in DPPH scavenger, which was concluded on the basis of comparing the absorbance values of sample with the absorbance values of positive control. From the above graph, it is shown that there is a rise in anti-oxidant activity from 20µl to 100µl concentration.

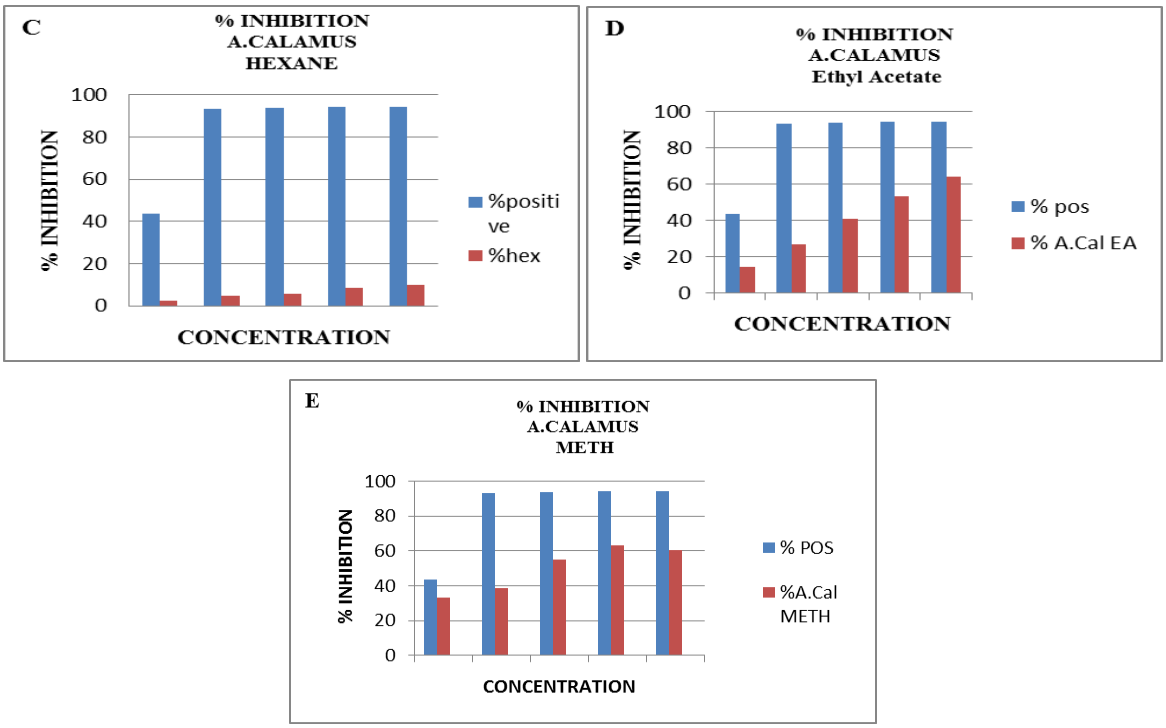


Fig6: The graphs C, D, E shows the DPPH activity of hexane, Ethyl Acetate, methanol extracts of A.calamus.

On comparing the graphs C, D and E, we conclude that Ethyl Acetate and methanol extract of A.calamus possess anti-oxidant activity greater than hexane. And also it is clear that for the rise in concentration there is a rise in anti-oxidant activity.

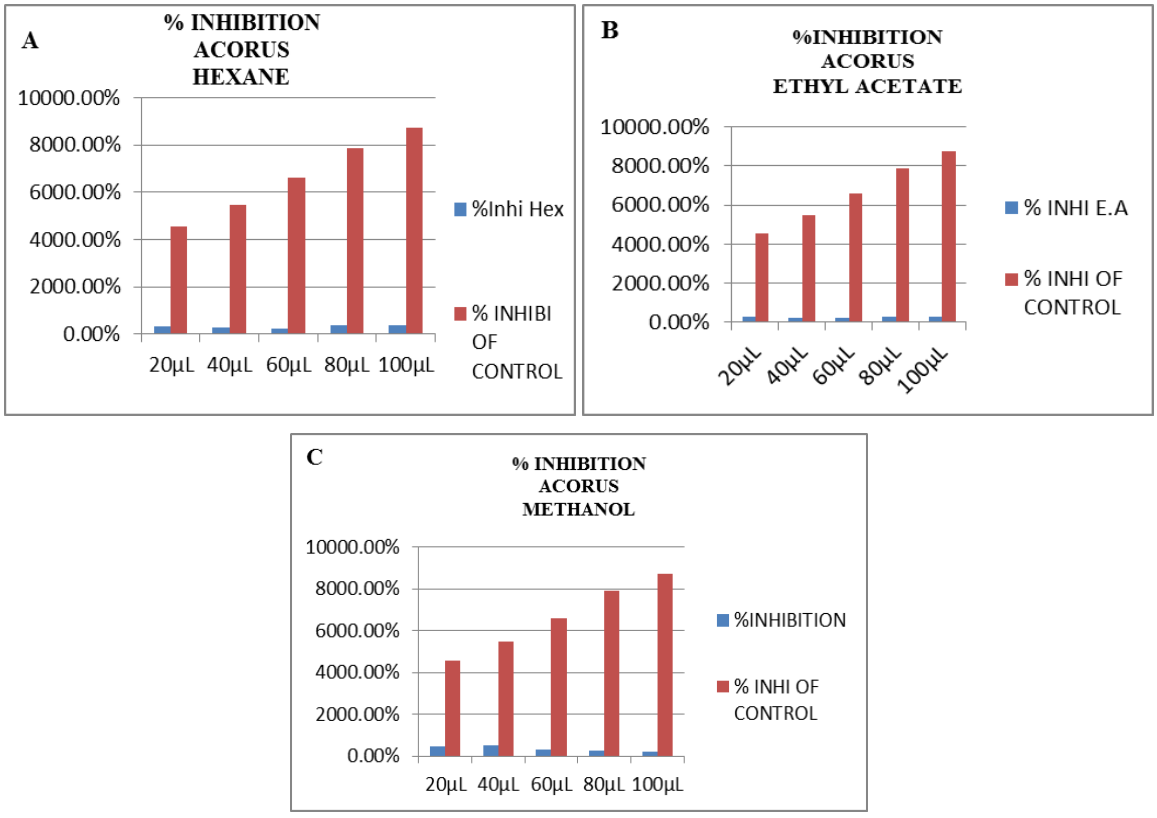


Fig7: A, B, C represent the anti-oxidant activity by H_2O_2 of hexane, ethyl acetate, methanol extracts of A.calamus

On comparing the above 3 graph we interpret that Hexane, E.A, methanol extracts possess very minimal concentration of anti-oxidant activity in H_2O_2 scavenger when compared with the absorbance of positive control values.

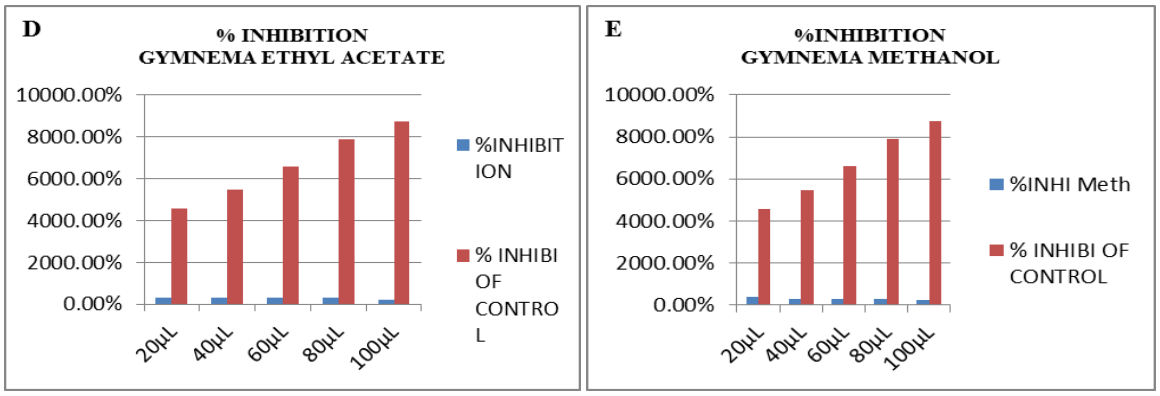


Fig8: Graph D and E represent the anti-oxidant activity of ethyl acetate and methanol extracts of G.sylvestre using H_2O_2 assay.

Both EA and methanol extracts of G.sylvestre also possess very minimal anti-oxidant activity by inhibiting H_2O_2 scavenger as like A.calamus.

Table: 1 Anti-bacterial activity of rhizome extract of acorus calamus.

Species	Extracts	Concentration	Zone of inhibition (mm)
Lactobacillus	**Hexane**	**Control**	**26mm**
		20 µL	13mm
		40 µL	14mm
		60 µL	17mm
		80 µL	18mm
	Ethyl acetate	**Control**	**27mm**
		20 µL	No zone
		40 µL	No zone
		60 µL	No zone
		80 µL	No zone
	Methanol	**Control**	**35mm**
		20 µL	23mm
		40 µL	24mm
		60 µL	25mm
		80 µL	27mm
Streptococcus mutans	**Hexane**	**Control**	**17mm**
		20 µL	15mm
		40 µL	16mm
		60 µL	18mm
		80 µL	20mm
	Ethyl acetate	**Control**	**32mm**

		20 µL	No zone
		40 µL	No zone
		60 µL	No zone
		80 µL	No zone
	Methanol	**Control**	**35mm**
		20 µL	No zone
		40 µL	No zone
		60 µL	No zone
		80 µL	No zone
	Hexane	**Control**	**35mm**
		20 µL	No zone
		40 µL	No zone
		60 µL	No zone
		80 µL	No zone
	Ethyl acetate	**Control**	**5mm**
		20 µL	No zone
Klebsilla		40 µL	No zone
		60 µL	No zone
		80 µL	No zone
	Methanol	**Control**	**35mm**
		20 µL	No zone
		40 µL	12mm
		60 µL	17mm
		80 µL	18mm
Pseudomonas aerugenisa	**Hexane**	**Control**	**1 mm**
		20 µL	No zone
		40 µL	No zone
		60 µL	No zone
		80 µL	No zone
	Ethyl acetate	**Control**	**34mm**
		20 µL	No zone
		40 µL	No zone
		60 µL	No zone
		80 µL	No zone
	Methanol	**Control**	**35mm**
		20 µL	No zone
		40 µL	No zone
		60 µL	17mm
		80 µL	25mm

Zone of inhibition is measured in mm at all concentration for all species tested. Some species does not possess anti-bacterial activity against A.Calamus, thus does not possess zone of inhibition.

Table: 2 Anti-Bacterial activity of leaves extract of Gymnema Sylvestre.

Species	Extracts	Concentration	Zone of inhibition (mm)
1. Lactobacillus	**Hexane**	**Control**	**25mm**
		20 µL	No zone
		40 µL	No zone

		60 µL	13mm
		80 µL	14mm
	Ethyl acetate	**Control**	**25mm**
		20 µL	No zone
		40 µL	13mm
		60 µL	23mm
		80 µL	27mm
	Methanol	**Control**	**26mm**
		20 µL	No zone
		40 µL	17mm
		60 µL	17mm
		80 µL	18mm
2. Streptococcus mutans	**Hexane**	**Control**	**15mm**
		20 µL	10mm
		40 µL	12mm
		60 µL	12mm
		80 µL	12mm
	Ethyl acetate	**Control**	**32mm**
		20 µL	No zone
		40 µL	No zone
		60 µL	No zone
		80 µL	No zone
	Methanol	**Control**	**37mm**
		20 µL	No zone
		40 µL	No zone
		60 µL	No zone
		80 µL	No zone
3. Shigella	**Hexane**	**Control**	**18mm**
		20 µL	No zone
		40 µL	No zone
		60 µL	No zone
		80 µL	No zone
	Ethyl acetate	**Control**	**10mm**
		20 µL	No zone
		40 µL	No zone
		60 µL	No zone
		80 µL	No zone
	Methanol	**Control**	**9mm**
		20 µL	No zone
		40 µL	No zone
		60 µL	No zone
		80 µL	No zone
4. Enterococcus facealis	**Hexane**	**Control**	**40mm**
		20 µl	No zone
		40 µL	No zone
		60 µL	No zone
		80 µL	14mm
	Ethyl acetate	**Control**	**25mm**
		20 µL	No zone
		40 µL	No zone
		60 µL	No zone
		80 µL	No zone
	Methanol	**Control**	**26mm**

		20 µL	No zone
		40 µL	No zone
		60 µL	No zone
		80 µL	15mm

The anti-bacterial activity of Gymnema Sylvestre were tested on the bacterial species above mentioned were listed with their anti-bacterial activity measurements in means of millimetre of zone of inhibition.

DISCUSSION

Guo-bing Shi et at investigated wound healing activity in the aqueous root and rhizome extract of Acorus using in vivo excitional wounding test. Through which he interpret that, root and rhizome of Acorus possess wound healing activity which was also determined by histological analysis. They also observed a significant healing of the wound in scarified mouse within 3 to 13 days due to the administration of aqueous extract of rhizomes and roots of A.calamus. In our research, methanol extract of Acorus calamus possess wound healing activity at both 100 and 200µl concentration (fig2) using scratch wound healing assay and ethyl acetate leaves extract of Gymnema sylvestre also possess wound healing activity at 100 and 200µl concentrations. P.V.Diwan et al has tested for anti-inflammatory activity in aqueous leaves extract of Gymenma sylvestre. They found that leaves of G.Sylvestre possess anti-inflammatory activity by inhibiting paw oedema in carrageenan-induced rat. Thus, he states that leaf of G.sylvestre has anti-inflammatory activity. Likewise, all the leaves extracts (hexane, E.A, methanol) of Gymnema sylvestre possess nearly 95% of anti-inflammatory activity, which was interrupted from HRBC assay (fig.3). Guo-bing Shi et al performed RT-PCR technique to detect anti-inflammatory activity in lipopolysaccharide induced RAW cell line. Through which he says that aqueous root and rhizome extract of A.calamus exhibit anti-inflammatory activity by inhibiting mRNA expression in lipopolysaccharide induced cell line. With this we state that rhizome extract of Acorus calamus showed drastic anti-inflammatory activity in all extracts at all concentrations (200-1000µg) (fig.4). P.R.Rachh et al states that alcoholic leaf extract of G.sylvestre shows significant anti-oxidant activity in DPPH and H2O2. With this we found that leave extract (hexane, E.A, Methanol) of Gymenma sylvestre possess anti-oxidant activity in both DPPH and H_2O_2 assays. Among which 80 and 100µL concentration showed significant anti-oxidant activity in DPPH assay whereas, in H_2O_2 assay E.A, methanol extract of G.sylvestre exhibits minimal concentration of anti-oxidant activity at all concentrations (200-1000µg). Under S. G. Funde's investigation in acetone and ethyl acetate extracts of Acorus calamus, he found that both the extracts of

Acorus possess anti-oxidant activity, which was interpreted by FRAP assay. He also states that in DPPH assay, methanol, ethanol and propanol extracts showed anti-oxidant activity in Acorus calamus. To this we state that rhizome hexane, E.A and methanol extracts of Acorus calamus showed anti-oxidant activity by inhibiting the scavenging activities of DPPH and H_2O_2 at all concentrations.

Asha and Deepak states that, ethyl acetate leaves and rhizome extract of A.calamus showed significant anti-bacterial activity against *E.Coli, pseudomonas aeruginosa, enterococcus faecalis, staphylococcus aureus, shigella sonnei, salmonella parathypi*. We have interpreted that hexane extract of A.calamus possess anti-bacterial activity by resisting the growth of *Lactobacillus* (18mm) and *streptococcus mutans* (20mm) in our research. Whereas, the growth of *Klebsilla* and *Pseudomonas Aeruginosa* were not inhibited by hexane extract of A.Calamus. E.A extract of A.Calamus does not exhibit anti-bacterial activity against *Klebsilla*, *Pseudomonas Aeruginosa*, *Streptococcus Mutans* and *Lactobacillus*. Wherein, methanol extract of A.calamus shows highest anti-bacterial activity on *Lactobacillus* (27mm), *Klebsilla* (18mm), and *Pseudomonas Aeruginosa* (25mm). Meanwhile, it does not shows significant anti-bacterial activity on *Streptococcus Mutans.* (Table: 1). Khanna and kannabiran states that saponin fractions of leaves extracts of Gymenma sylvestre showed anti-bacterial activity against various organism like *E.Coli, S.typhi, S.aureus, K.pneumoniae and P.mirablis*. We concluded that hexane, E.A and methanol extracts of G.sylvestre possess anti-bacterial activity on *Lactobacillus*. Growth of *Enterococcus Facealis* and *Shigella* was not inhibited by Hexane, E.A and methanol extracts of Gymnema sylvestre. Whereas, only hexane extract of G.sylvestre shows significant anti-bacterial activity against *Streptococcus Mutans.* (Table 2).

CONCLUSION

Therefore, our research states that rhizome and leave extract of Acorus Calamus and Gymnema Sylvestre respectively possess wound healing, anti-inflammatory, anti-oxidant and anti-bacterial activity at various concentrations with various solvents like hexane (low polarity), ethyl acetate (mid polar), methanol (high polarity). At 200 µL the wound healing activity of methanol extracts of both Acorus and Gymnema is higher when compared to 100 µL. Likewise, both Acorus and Gymnema possess anti-inflammatory at 10, 30 and 50 µL, in addition to this, it is also clear that both Acorus Calamus and Gymnema Sylvestre possess anti-inflammatory activity at higher concentration. Anti-oxidant activity was evaluated using

DPPH assay in which 100 µL concentrations of Gymnema extracts of ethy acetate and methanol possess 65% and 75% (approximately) anti-oxidant activity, whereas, Acorus Calamus possess anti-oxidant activity in methanol and ethyl acetate extracts than hexane. It is also found that, there is a rise in % inhibition in accordance with the rise in rise in concentration. However in H_2O_2 assay for detecting anti-oxidant, there is only very minimal anti-oxidant activity found in all extracts of both Acorus as well as Gymnema. Hexane extract of Acorus Calamus has inhibited the growth of *Lactobacillus* and *Streptococcus mutans* and methanol extract of Acorus Calamus has inhibited the growth of *Klebsilla a*nd *Pseudomonas aerugenisa,* in addition to this, it is also clear that ethyl acetate extract of Acorus calamus does not possess anti-bacterial activity. Likewise, *lactobacillus, streptococcus mutans* and *enterococcus facealis* were not grown in hexane extracts of Gymnema sylvestre. *Lactobacillus* and *enterococcus facealis* growth were inhibited by methanol extract. Ethyl acetate extract has restricted the growth of *lactobacillus*. In addition to this, *Shigella* growths were not restricted by any of the extracts of Gymnema sylvestre.

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REFERENCE

1. P.V. Diwan, I. Margaret, S. Ramakrishna. Influence of Gymenma sylvestre on inflammation. Inflammopharmocology, 1995; 3: 271-277.
2. Venkatesan Gopiesh khanna, Krishnan Kannabiran. Antimicrobial activity of saponin fractions of the leaves of Gymnema sylvestre and Eclipta prostrate. World J Microbiol Biotechnol, 2008; 24: 2737–2740.
3. Antony Gomes, Rinku das, Sumana Sarkhel, Roshnara Mishra, Sangamithra Mukherjee, Shamik Bhattacharya et al. Herbs and herbal constituent's activity against snake bite. Indian journal of experimental biology, 2010; 48: 865-878.
4. Yanling Chen. Scratch Wound Healing Assay. Bio-protocol, 2012; 2.
5. P.R. Rachh et al. Invitro analysis of anti-oxidant of Gymnema sylvestre R. BR. Leaf extract. ROM. J. BIOL. – PLANT BIOL, 2009; 54: 141-148.
6. Asha Devi S and Deepak Ganjewala. Antimicrobial activity of Acorus calamus (L.) rhizome and leaf extract. Acta Biologica Szegediensis, 2009; 53(1): 45-49.

7. Abdollah ghasemi pirbalouti, Abed koohpayeh, Iraj karimi. The wound healing activity of flower extracts of *punica granatum* and *achillea kellalensis* in wistar rats. Acta Poloniae Pharmaceutica-Drug Research, 2010; 67(1): 107-110.
8. Durbadal Ojha, Hemanta Mukherjee, Supriya Mondal, Aditya Jena, Ved Prakash Dwivedi,Keshab C. Mondal et al. Anti-Inflammatory Activity of Odina wodier Roxb, an Indian Folk Remedy, through Inhibition of Toll-Like Receptor 4 Signaling Pathway. PLoS ONE, 2014; 9(8).
9. Mahmood, A.A., K.Sidik, I.Salmah. Wound healing activity of *carica papaya* L. Aqueous leaf extract in rats. International journal of molecular medicine and advance sciences, 2005; 1(4): 398-401.
10. I.J.Kuppast, P.Vasudeva Nayak. Wound healing activity of *Cordia Dichotoma* Forst. f. Fruits. Natural product radiance, 2006; 5(2): 99-102.
11. Bulti nayak, satarupa roy, madhumita roy,A.Mitra, kalpana karak. Phytochemical, Antioxidant and Antimicrobial Screening of *Suaeda maritima* L (Dumort) against Human Pathogens and Multiple Drug Resistant Bacteria. Indian Journal of Pharmaceutical Sciences, 2018; 26-35.
12. Murugan M, Mohan V. R and Thamodharan V**.** Phytochemical screening and antibacterial activity of *Gymnema sylvestre* (Retz) R. Br ex. Schultes and *Morinda pubescens* J.E.Smith var. *pubescens.* Journal of Applied Pharmaceutical Science, 2012; 02(02): 73-76.
13. Shanbhag tara v., Sharma Chandrakala, Adiga Sachidananda, Bairy Laxminarayana Kurady, Shenoy smita and Shenoy ganesh. Wound healing activity of alcoholic extract of *kaempferia galanga* in Wistar rats. Indian J Physiol Pharmacol, 2006; 50(4): 384–390.
14. Amoolya Gowda, Venkatesh Shanbhag, Smita Shenoy, Eesha Rao Bangalore, Krishnananda Prabhu, Raghu Murthy et al. Wound healing property of topical application of ethanolic extract of *Michelia champaca* flowers in diabetic rats. International Journal of Pharmacology and Clinical Sciences, 2013; 2(3): 67-74.
15. M. A. Ekpo and P. C. Etim. Antimicrobial activity of ethanolic and aqueous extracts of *Sida acuta* on microorganisms from skin infections. Journal of Medicinal Plants Research, 2009; 3(9): 621-624.
16. Guo-bing Shi, Bing Wang, Qiong Wu, Tong-chao Wang, Chang-li Wang, Xue-hui Sun et al. Evaluation of the wound-healing activity and anti-inflammatory activity of aqueous extracts from *Acorus calamus* L. Pak. J. Pharm. Sci., 2014; 27(1): 91-95.

17. S. G. Funde. Phytochemicals evaluation, anticancer, antioxidant and antimicrobial activity of *Acorus calamus* different solvent extracts. Journal of Chemical and Pharmaceutical Research, 2015; 7(6): 495-504.
18. P. Rajasulochana, R. Dhamotharan, P. Krishnamoorthy. Primary phytochemical analysis of *kappaphycus sp*. Journal of American Science, 2009; 5(2): 91-96.
19. P Rajasulochana, P Krishnamoorthy and R Dhamotharan. An investigation on the antioxidants, antifungal and antibacterial of the *kappaphycusalvareziI*. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2013; 4(1): 586–594.
20. P. Rajasulochana, R. Dhamotharan, P. Krishnamoorthy, S. Murugesan. Anti-bacterial activity of the extracts of marine red and brown algae. Journal of American Science, 2009; 5(3): 20-25.