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Research Article

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Phytochemicals evaluation, anticancer, antioxidant and antimicrobial activity of *Acorus calamus* different solvent extracts

S. G. Funde

Dr. Shree nanasahebDharmadhikari Arts, Commerce and Science College, University of Mumbai, Gove kolad, Tal-Roha, Dist- Raigad Maharashtra, India

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ABSTRACT

Acorus calamus has been used for centuries as a folk medicine to treat infectious and noninfectious diseases. Extracts of dried acorus prepared with different solvents, (acetone and ethyl acetate) were tested for Phytochemical analysis, antioxidant, antimicrobial, antioxidant, anticancer and dna polymerase inhibitory activity. Phytochemical analysis revealed that acetone extracts contains total polyphenolic and flavonoids 10.00 and 0.725 mg/gm where as tannic acid (35.55), Pyrogallic acid(694.8), and Querctin (428.8) ug/gm. Antioxidant activity of acetone and ethyl acetate crude extracts by FRAP are 47.03 and 30.09Mm FeSO4 / gm of sample. Acetone extract is having potent antimicrobial activity against S.Typhi, Ecoli and M. Luteus are 47, 43.85, 100 % inhibition at 13 ug conc. of extract. Complete DNA polymerase inhibition observed at 6.66 ppm conc. of acetone extract where no inhibitions observed in a ethyl acetate extract. Acetone extract is having high HT – 29 cell proliferation inhibition activities (53.44% inhibition) 10 ug of extracts. Acorus calamus is beneficial for the future multi diseases therapeutic research study.

Key words: *Acorus calamus*, anticancer, crude drug, antimicrobial activity, natural product

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INTRODUCTION

WHO report indicates the major need of drug discovery for infectious (pathogenic) and non-infectious diseases (CVD, Cancer, Alzheimer etc.). To discoverer new drug for single disease is very critical and complicated and time consuming process. But drug discovery on the basis of traditional medicinal system of medicinal plant is might be a short term process (1, 2). Large numbers of the medicinal plant are used for the treatment of various diseases as compare to allopathic medicine. Although drug discovery from medicinal plants continues to provide an important source of new drugs but it leads, numerous challenges are encountered including the procurement of plant materials, selection and implementation of appropriate high-throughput screening bioassays, and scaling up active compounds (2, 3).

Acorus calamus is one of the medicinal plants observed in Asia, North America and Europe. It is widely used for the formulation of medicine in chineases and Indian ayurveda. Different plant part is used in formulation of the ayurvedic medicine. Rhizome use in emetic, kil lice nervous system, throat infection diarrheal, smallpox, respiratory ,gastrointestinal track ,gout rheumatism and mental disorder treatment. Leaves used wound to kil lice and stem for cough, cold, and toothache. Root and bark is used as antidotes for snake bite. *Acorus calamus* plant material different type of extracts had showed different activity was Like alcoholicand other solvent extracts had anti-inflammatory, CNS depressant behavioral changes, anticancer, antiulcer, cytoprotective, hypolipidenic, antimicrobial, Antioxidant, acetylcholine inhibitory activity (3,4,5,6). Aqueous extract antidiharial behavior

changes, CNS depressant hypnotic effect aqueous-alcohol combined solvent extract have hypolipedimic activity in rat (7, 8, 9, 10). This study is aimed to investigate the effect of different solvent extractions on phytochemicals and different activities like in vitro DNA polymerase inhibitory activity, anticancer, antioxidant and antimicrobial activities.

EXPERIMENTAL SECTION

2.1 Chemicals

Methanol (MeOH), Ethanol (EtOH), Propanol(PrOH), Butanol(BuOH), Ethyl acetate(EtOAc), Acetone(Act) and Acetonitrile (ACNT) Qualagen. Type I agar and Luria Britannia broth, (Himedia) Quercetin, Caffeic acid, Catechin, Gallic acid, Ascorbic acid(AA), Hydrochloric acid(HCl), Ferrous sulphate(FeSO4), Sulfuric acid(H2SO4), Sodium phosphate, Ammonium molybdate, Ammonium ferrous sulphate (NH3(FeSO4)), Diphenyl picryl hydrazine (DPPH), Ferric chloride(FeCl3),Folin ciocalteu reagent(FC reagent), (TPTZ), pyrogallic acid, tannic acid, hydraquinon, vanillin, quercetin (Germany). Methanol (HPLC gradient grade) Merck (Germany).Orthophosphoric Acid (OPA), RANKEM, Acetic acid (HPLC grade) was purchased from J.T. Baker (Netherlands). Milli-Q water was used in all experiments. Microbial strain *Ecoil 25922 (ATCC), Basillus subtillus 2063 (ATCC), Styphylococcus epidermidis12228 (ATCC), Styphylococcus aureus 25923 (ATCC), Micrococus luteus 9341(ATCC)and Psedomonas aeroginosa 27853 (ATCC) was* purchased from national chemical laboratory Pune, India. HT29 cell line (obtained from NCCS, Pune) was used for study of cell proliferation study.

2.2 Extraction

*Acorus calamus*whole plant 1gmpowder was extracted with 15 ml various solvents like methanol, ethanol, propanol, ethyl acetate, acetone, acetonitrile and water at 37 ^{0}C with shaking condition. Sample prepration for biological study vrious extract dried under nitrogen atmosper and dissolved in DMSO.

2.3Determination of polyphenolic content

Total polyphenolic content was analyzed by the Folin–Ciocalteu method (11). The reaction mixture (3.6 mL) contains 20µL of sample, 3.2 ml of distilled water, 100µL of FC reagent; 300 µL of 60µg of saturated sodium carbonate was incubated at 37^{0}C for 30 min in water bath the absorbance was read at 765 nm was measured in triplicate. Gallic acid (100µg/mL) was used for calibration of standard curve. The results were expressed as mg of gallic acid equivalent (mg GAE)/g of dry plant material.

2.4 Chromatographic analysis of polyphenolic and flavonoids

A simple and quick reversed phase HPLC method used for determination of phenolic acids (12– 13). Chromatographic analysis was with the use of liquid chromatographic system, which consisted of P680 HPLC Pump, ASI-100 manual sample injector, thermostat column compartment C18, UVD170U detector. Chromatographic system was connected through the water universal chromatography interface to the computer. Software used for data acquisition and evaluation was Water. The separation was carried out on inertsil ODS 3V, 250*4.6*5 micron reversed phase column. Column temperature was maintained at 40 °C. Elution was performed by using gradient with the mobile phase consisting of mixture of buffer and acetonitrile (buffer 0.1M KH2PO4, PH 3.5 with OPA). Mobile phase A (buffer: ACN = 900:100), Mobile phase B (buffer: ACN = 450:550) and the flow rate was 1 ml/min, with following gradient program (Table 2.1).

Table 1 – Gradient program of solvent system for HPLC

Time in Min	Mobile Phase A	Mobile Phase B
00	95	05
08	95	05
20	50	50
35	44	56
45	44	56
50	10	90
75	10	90
80	95	05
90	95	05

The injection volume for all samples was 20µL. For detection, chromatograms were monitored at 275 nm. Identification of phenolic acids was based on retention times in comparison with standards. The quantification was

carried out using the external standard method. Stock solution of standard compounds at concentration 1 mg/ml each was prepared in methanol. The solution of standards was injected into the HPLC system.

2.5Antioxidant Activity

2.5.1 Total antioxidant potential

Total antioxidant potential of crude extract was determined by the Phosphomolybdate method (15). Final volume of the reaction 3.010 ml containing 3.0ml of (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) 10 µL of the respective extract incubated at 95^0C for 90 min. After the samples were cooled at room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank which contains 10 µL of extracting solvent. Antioxidant potential of extracts were expressed as mM of Vit. C /g of dry plant material. The estimation was carried out in triplicate.

$$\text{mg of Polyphenolics} = \frac{(\text{O. D. of Standard} - \text{O. D. of Sample})}{(\text{O. D. Standard})} \times \text{Conc. of Stdandard}$$

2.5.2 Ferric-reducing antioxidant potential assay

The FRAP assay was carried out according to the procedure of Benzie and Strain(16) with modification. FRAP reagent was prepared from sodium acetate buffer (300 mMol/L, pH 3.6), 10 mMol/L TPTZ solution in 40 mMol/L HCl and 20 mMol/L $FeCl_3$ in 40 mMol/L HCl solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was stored in cooled condition at 0-4 ^{0}C for five days. Before use the reagent was warmed to 37 °C in a water bath. 25µL of each extracts was added to 1.475 mL of sodium acetate buffer and 1.5 mL of FRAP reagent. The absorbance of the reaction mixture was then recorded at 593 nm after 5 min. The standard curve was constructed using $FeSO_4$ solution (10–100 µMol/L). The results were expressed as µMol Fe (II)/g dry weight of sample.

$$\mu\text{M of Fe(II)} = \frac{(\text{O. D. of Standard} - \text{O. D. of Sample})}{(\text{O. D Standard})} \times \text{Conc. of Standard}$$

2.5.3 Free radical scavenging ability by DPPH radical

The DPPH assay was carried out by 96 well plate method (16). DPPH assay is based on the measurement of the scavenging ability of antioxidants towards the stable DPPH radical. The free radical DPPH, which shows absorption at 517 nm, is reduced to the corresponding hydrazine when it reacts with hydrogen donors. Stock solution of DPPH 1 mM was prepared in methanol and working solution 0.1mM prepared in methanol. 10 µL of 1:4 diluted plant extract was added in 100 µL of methanol and 100 µL of 0.1mMol of DPPH. The mixture was shaken and allowed to stand at room temperature in the dark for 10 min. The decrease in absorbance of the resulting solution was monitored at 517 nm at 10 min. The results were expressed in % of inhibition by following formula.

$$\% \text{ of inhibition} = \frac{(\text{O. D. of control} - \text{O. D. of sample})}{(\text{O. D control})} \times 100$$

2.6Taq polymerase inhibition assay

Polymerase inhibition assay were contains final reaction volume 25.2 µL In brief reaction contains addition of 17.5 µL of distilled water 2.5 µL of AccuTaqg polymerase buffer 0.5 µL of Template DNA 2.5 µL dNTPs 1 µL of forwarding primer, 1 µL of reverse primer and 0.2 µL AccuTag polymerase such reaction is subjected for PCR. The PCR program is set as above Intial denaturation at 95 °C for 5 min. 35Cycle (such as denaturation at 95 sec annealing 62°C fro 45 sec extension at 72°C for 1 min) final extension at 72°C for 5 min and hold at 20°C for ∞.

How ever methanol is used as positive controls. After PCR reaction 15 µL aliquot of the mixture from reaction was loaded into 1.0% agarose gel containing ethidium bromide (0.05 µg/mL) in Tris-acetate-ethylenediaminetetraacetic acid (EDTA) buffer. The electrophoresis was carried out for 30 min at 100 V, and then gels were illuminated with UV light and photographed

2.7 Antimicrobial activity

Susceptibility testing against *S.areus*, *Bacillus subtilis* and *Ecoli* was performed using turbidometric methods (18,19). Samples (dissolved in DMSO) were serially diluted using 0.9% saline and transferred in qudriplate to 96-well microplates. Microbial inocula were prepared after comparison of the absorbance at 600 nm of cell suspensions to diluting the suspensions in broth to afford recommended inocula. Microbial inocula were added to the diluted

samples to achieve a final volume of 200 µL. Growth, solvent, and media controls were included in each assay. Plates were read at 600 nm prior to and after incubation. Antimicrobial activity was calculated by using following formula

$$\% \text{ of inhibition } = \frac{(\text{O. D. of Control} - \text{ O. D. of Sample})}{(\text{O. D. Control})} \times 100$$

2.8 Anticancer assay

Cytotoxicity was estimated by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assays as described previously (19,20). The cell line HT-29 (colorectal adenocarcinoma cell line from Human colon) procured from NCCS, Pune (India). These cells were grown as adherent culture in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% foetal bovine serum and Ciprofloxacin (10µg/ml) at 37°C in a humidified 5% CO_2 incubator.

For cytotoxicity screening, cells were seeded in 96-well microplates at a density of 1×10^5 cells per well to which extracts (DMSO) at the final concentration of 15 µg were added in quadruplicates and incubated for 24 hours. The intensity of the purple colour (formazan) was measured on a microplate reader at 570 nm to determine cell viability. The percentage of inhibition was determined by comparing cell viability in absence and presence of extracts.

$$\% \text{ of inhibition } = \frac{(\text{O. D. of Control} - \text{ O. D. of Sample})}{(\text{O. D. Control})} \times 100$$

RESULTS

3.1 Total Phenolic Contents

The total phenolics in crude extracts of *Acorus calamus* were expressed as gallic acid equivalent. As shown in Table 2, the total phenolic content in methanol, ethanol and acetoneextract showed the highest amount, followed by propanol and acetonitrile.

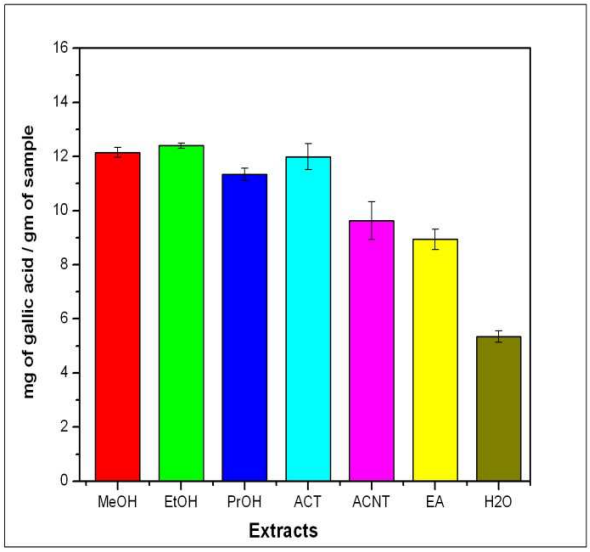


Fig – 1 Total Phenolic Content of A. calamus extracts

MeOH- methanol extract, EtOH – ethanol extract, PrOH – propanol extract, ACT – acetone extract, ACNT- acetonitrile extract, EA – ethyl acetate extract and H2O - water extract; Data are shown as mean±S.D. one way ANOVA p = < 0.001 which represents the significance, n (sample size) = 3.

3.2Polyphenolics and Flavonoids

Table 4.2- Estimation of phytochemicals of *Acoruscalmus* by using HPLC method

Phytochemicals	MeOH µ/gm of sample	EtOH µ/gm of sample	PrOH µ/gm of sample	ACT µ/gm of sample	ACNT µ/gm of sample	EA µ/gm of sample	H_2O µ/gm of sample
Chlorogenic acid	2278	1157.10	1793.05	2123.1	491.56	132	1698.54
Epigallactocatechin	36.04	20.576	39.27	ND	11.088	ND	ND
Curcumin	50.88	48.57	30.76	50.44	706.05	523.5	240.22
Gallic acid	1962.1	1872.64	2728.25	ND	2833.05	ND	97.8
Quercetin	672.52	602.03	591.5	519.4	427.92	11.299	70.49
Naringin	700.39	627.46	615.96	541.34	445.99	11.76	73.46
Naringenin	4.607	ND	4.725	ND	ND	2.344	16.416
Rutein	207.34	88.64	282.43	74.34	43.32	ND	220.78

MeOH- methanol extract, EtOH – ethanol extract, PrOH – Propanol extract, ACT – acetone extract, ACNT- acetonitrile extract, EA – ethyl acetate extract and H_2O – water extract. ND – not determined.

Maximum extraction of the chlorogenic acid is observed in the Propanol and water extract 1.793 and 1.69 mg / gm of sample. Acetonitril, ethyl acetate and water extracts contains maximum amount of curcumin compounds shown in table- 2. Higher extraction of the quercetin and naringenin flavonoids are observed in the alcoholic, acetone and acetonitril extracts where as maximum extraction of the rutein and naringn flavonoids is observed in water and alcoholic extracts Table 2 Presence of the flavonoids and other polyphenolics compounds are responsible for the antioxidant activity, antibacterial, antiviral, antiinsecticidal etc. activities (21– 26).

3.3Antioxidant Activities

3.3.1 RadicalScavengingActivity

DPPH radical scavenging activity of crude extracts of *Acorus calamus* is shown in Fig –2. The highest activity was observed in the methanol, propanol and ethanol extracts, whileacetonitril and water extract also showed good inhibitory effects. DPPH radical inhibition by *Acorus calamus*extracts is decreased in the following order: MeOH> EtOH = PrOH> ACNT> H_2O >ACT = EA .These results indicate that *Acorus calamus*alcoholic extracts have better performance against DPPH radical scavenging activity than the other solventextracts.

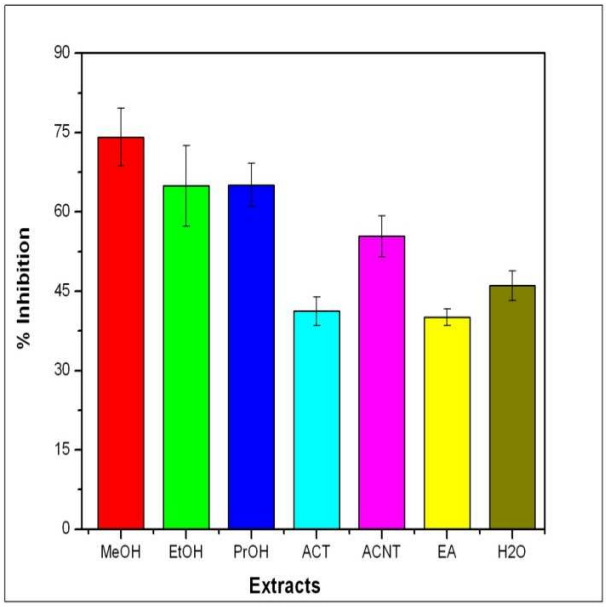


Fig. – 2 Radical scavenging activity of different solvent extracts

MeOH- methanol extract, EtOH – ethanol extract, PrOH – Propanol extract, ACT – acetone extract, ACNT- acetonitrile extract, EA – ethyl acetate extract and H_2O - water extract. Data are shown as mean±S.D. one way ANOVA p = < 0.001 which represents the significance, n (sample size) = 3.

3.3.2 ReducingPower

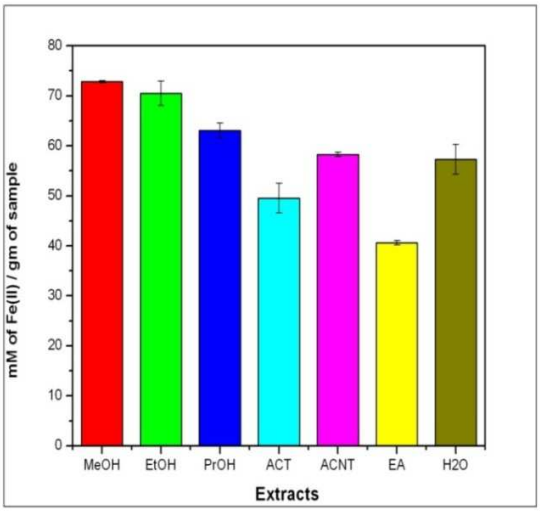


Fig – 3 Metal reducing antioxidant activity of different extracts

MeOH- methanol extract, EtOH – ethanol extract, PrOH – Propanol extract, ACT – acetone extract, ACNT- acetonitrile extract, EA – ethyl acetate extract and H_2O - water extract. Data are shown as mean±S.D. one way ANOVA p = < 0.001 which represents the significance, n (sample size) = 3.

The reducing power of extracts serves as a significant indicator for its potential antioxidant activity. In this assay, the color of the test solution changes to blue depending on the reducing power of test extracts. The presence of reductants in the solution causes the reduction of the Fe^{3+}/Fe^{2+} form (15). The antioxidant activity of *Acorus calamus* L extracts in vitro is shown in Fig –3. Of the extract, Alcoholic extracts show maximum antioxidant activity. Other extracts also have markeble antioxidant activity.

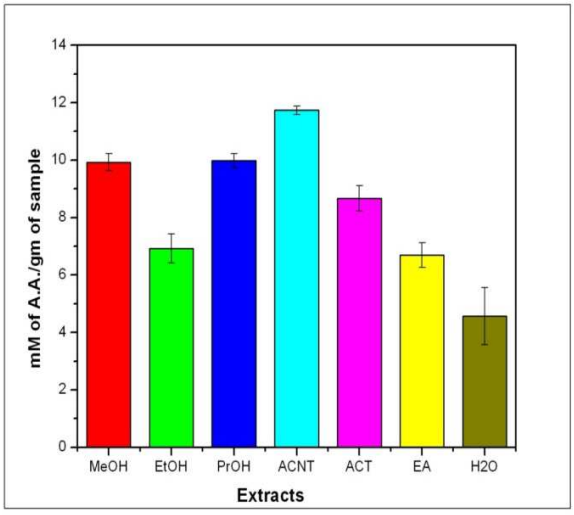


Fig – 4phosphomolybdate antioxidant activity of different extracts

MeOH- methanol extract, EtOH – ethanol extract, PrOH – Propanol extract, ACT – acetone extract, ACNT- acetonitrile extract, EA – ethyl acetate extract and H_2O - water extract. Data are shown as mean±S.D. one way ANOVA p = < 0.001 which represents the significance, n (sample size) = 3.

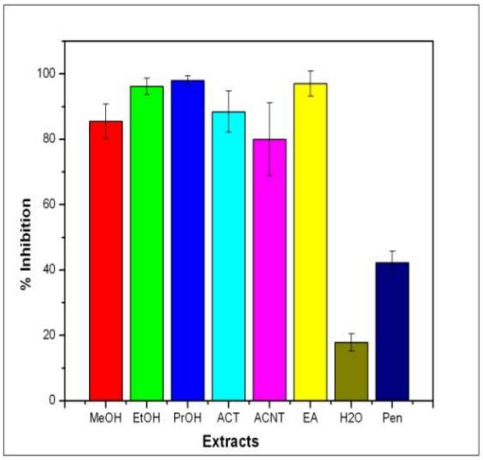
3.2.3 Phosphomolybdate antioxidant

All extracts showed the molybdate reducing power. Amoungst that acetonitril, propanol and methanol extract (11.737, 9.98 and 9.926 mM of ascorbic acid/ gm of sample) showed higher than that of ethanol, acetone and water

extract (6.922, 8.669 and 4.568 mM of ascorbic acid/ gm of sample) Fig – 4. Effectiveness in reducing power was calculated with ascorbic acids values;

3.3 Antimicrobial activities

All extracts were evaluated in vitro for antibacterial activities against *Stphylococcusepidermidis*, *Stphylococcusaureus* and*Micrococusluteus* by turbidometric assay (18, 19). Most of the *Acorus calamus* extracts showed moderate to mild activities with % inhibition ranging from 0 to 99% inhibition. Fig. 5, 6,and 7. Alcoholic and other extracts shows potent activity on *Stphylococcusepidermidis*. The most potent antimicrobial activity was exhibited by alcoholic extracts, which contains higher amount of naturally occurring antimicrobial compounds. Over all alcoholic extracts found to be most active against *Stphylococcusepidermidis*, *Stphylococcusaureus* and*Micrococusluteus* as compared to acetone, acetonitrile, ethyl acetate and water extracts. Water extract is not able to inhibit the micrococcus luteus and Staphylococcus aureus organism.



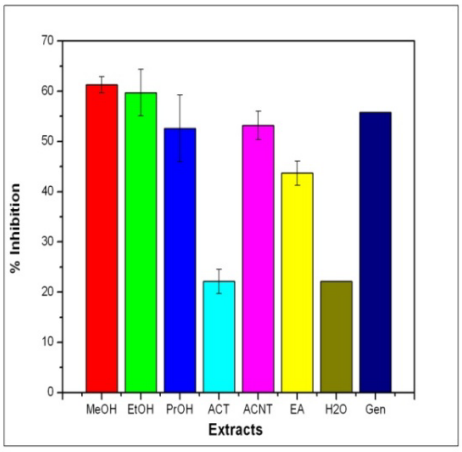


Fig – 5, Fig - 6 Growth inhibition activity against *S. Epidermis* and *S. aureus*

MeOH- methanol extract, EtOH – ethanol extract, PrOH – Propanol extract, ACT – acetone extract, ACNT- acetonitrile extract, EA – ethyl acetate extract and H_2O - water extract (2 µL conc. of extract was used for expt.). Pen and Gen are standard 50 ng antibiotic for S. aureus and S. epidermis. Data are shown as mean±S.D. one way ANOVA p = < 0.001 which represents the significance, n (sample size) = 4.

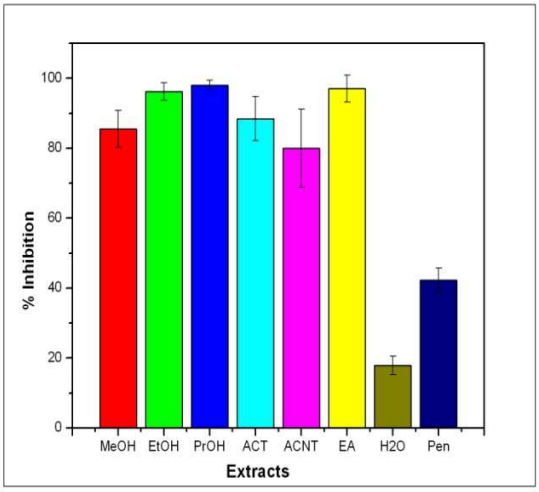


Fig – 8 Growth inhibition activity against *M. luteus*

MeOH- methanol extract, EtOH – ethanol extract, PrOH – Propanol extract, ACT – acetone extract, ACNT- acetonitrile extract, EA – ethyl acetate extract and H_2O - water extract (2 µL conc. of extract was used for expt.) . Pen is standard 50 ng antibiotic for M. luteus. Data are shown as mean±S.D. one way ANOVA p = < 0.001 which represents the significance, n (sample size) = 4.

3.4 Cancer cell proliferation activities

The anticancer activity of the extracts was investigated using MTT assay on human colon cancer cell line HT-29 (26). A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells (26). Fig –9show that methanol, ethanol, propanol, extracts significantly inhibited cancer cell growth (HT-29 cell lines) compared to acetone, acetonitrile and ethyl acetate extracts. At a concentration of 15 µg/mL, the percentage of anticancer activity of ethanol, propanol, acetonitrile and methanol extracts (66.56, 63.50, 62.14, 61.32%), was found significantly higher than acetone and ethyl acetate extracts(46.12 and 49.89%). The negative activities of Water extract to HT-29 cell lines which did not inhibit cancer cell growth, but apparently increased the growth rate of cancer cells. However, more confirmative studies will be essential in the future.

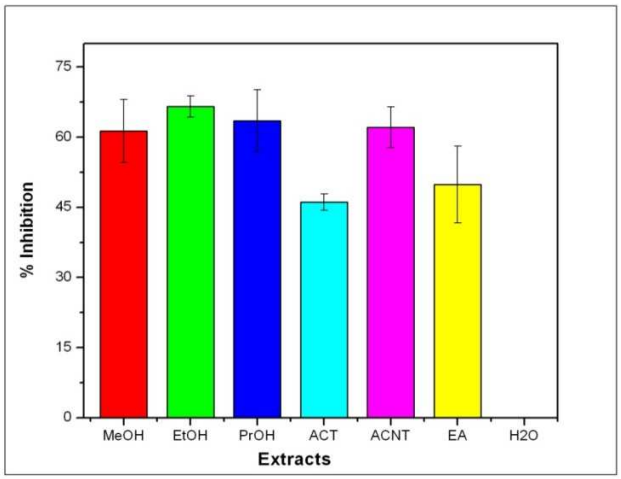


Fig – 9 Growth inhibition activity on HT -29 cell lines

MeOH- methanol extract, EtOH – ethanol extract, PrOH – Propanol extract, ACT – acetone extract, ACNT- acetonitrile extract, EA – ethyl acetate extract and H_2O - water extract (5 µL conc. of extract was used for expt.). Data are shown as mean±S.D. one way ANOVA p = < 0.001 which represents the significance, n (sample size) = 3.

3.5 Polymerase inhibitions

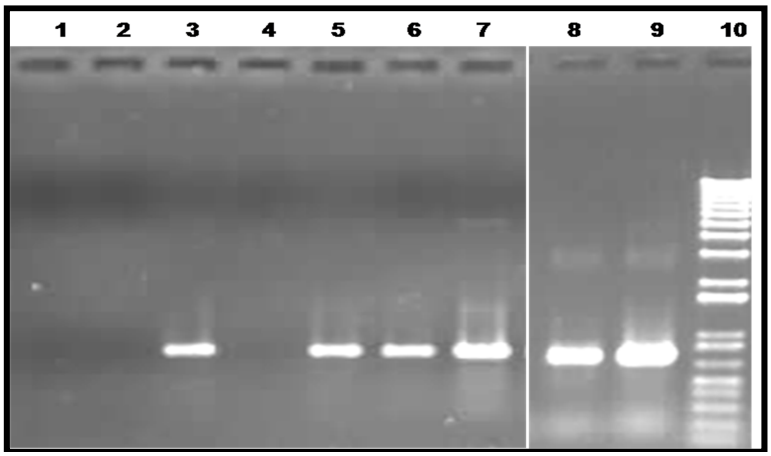


Fig 10 –Gel electrophoresis patteren of the polymerase inhibition activity of plant extracts

Well 1-8 contains respective presence of extracts [1 methanol extract, 2 ethanol extract, 3 ethyl acetate extract, 4 acetonitril extract, 5 acetone extract, 6 propanol extract and 7 water extract] 8-ve control, 9 +ve control, 10 Marker Extract used for assay - 2 uL from 1: 25 diluted extract

DNA polymerase is the major enzyme which is involved in the cell division process of eukaryotic, prokaryotic and viral cell. Fig 10shows the inhibition of different solvent extract of *Acorus calamus* on DNA polymerase. Polymerase activity was the most severely inhibited by thedifferent solvent extracts of *Acorus calamus* indicates that

methanol, ethanol and propanolextracts have complete polymerase inhibition while acetone and ethyl acetate extracts has inhibition.

DISCUSSION

Recent studies showed the presence of phytochemicals like flavonoids and phenolics in various extracts of the acorus. Alcoholic extractshad relatively high phenolic contents compared to other extracts. Phytochemicals extraction is varying from solvent to solvent used for extraction. Accordingly, anti-radical tests showed that higher polyphenolics extracts contains stronger radical-scavenging activity (DPPH). Alcoholic extracts has higer metal reducing property.

Conveniently, the antioxidant, antimicrobial, polymerase inhibition and anticancer property of a plant extract is generally considered as the result of the combined activity of a wide range of compounds, including phenolics, peptides, organic acids and other components (11, 27, 28).Our results suggest that acoruscalmus alcoholic extracts can be considered as a valuable source of biologically active products, especially of naringin, qunine, quercetin and chlorogenic acid. These compounds, in addition to its strong pharmacological activity such as antioxidant, acetylcholine inhibitor, antiviral, anti-microbial properties (28, 29), so that acoruscalmus might find a number of traditional medicnal applications.

Many studies have revealed that the phenolic contents in plants are related to their antioxidant activities. Antioxidant activities of phenolic compounds are probably due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (30, 31).

CONCLUSION

The clinical efficacies of many medicinal plant preparations used by ayurvedic practitioners are not yet validated. The mechanism of action of some of the identified natural products is known but as the active ingredients in many plants extracts possessing various properties remains to be identified. A further elucidation of both known and yet to be identified natural antioxidants clubbed with newly emerging technology, metabolomics could help disease prevention and cure using simple herbs.

REFERENCES

[1] DO Kennedy;ELWightman; *AdvNutr* **2011**, 2 , 32-50,
[2] GACordell; *Phytochemistry review* **2002**, 1, 261-273
[3] SMehrotra; KPMishra; RMaurya; RCSrimal; VSYadav; RPandey; VK Singh; *International Immunopharmacology*, **2003**, 3, 53-61
[4] VSPawar;AAkhade;SBaokar;HShivakumar; *Asian Pacific J. of Tropical Biomedicine*, **2011**, 1,S17-S19
[5] SMehrotra;SMehrotra;KPMishra;RMaurya;RCSrimal; R Pandey; VK Singh *International Immunopharmacology* **2003**, 3, 53–61
[6] SBVohora;SAShah; PCDandiya; *J. of Ethnopharmacology* **1990**, Vol . 28, Issue 1, pp 53–62
[7] NPujenjob;VRukachaisirikul;MOngsakul ; *J. Sci. Technol*. **2005**, 27, 518-523
[8] PK.Shukla; VKKhanna; M MAli; RRMaurya; SSHanda;RCSrimal; *Phytotherapy Research* **2002**, 16, 256–260,
[9] AMuthuraman ; NSingh; *BMC Complementary and Alternative Medicine* **2011**, 11:24
[10] TSSubha, AGnanamani ,ABMandal ; Pharamcognostic Evaluation of *Acorus calamus*.*Pharmacognosy Journal*, **2011**, 3, 24-27
[11] LRFukumoto;GMazza; *J. Agric. Food Chem.*, **2000**, 48, 3597–3604.
[12] B Klejdus;R Mikelova;JPetrloa;DPotesil;VAdam;MStiborova;PHodek;JVacek;RKizek;VKuban; *J. Chromatography A.* **2005**, 1084, 71–79.
[13] MARostagno;M Palma;CGBarroso; *AnalyticaChimicaActa*. **2007**, 582, 243–249.
[14] MPGermano, DER.Pasquale, VAngelo, SCatania, VSilvaria, CCosta ; *J. Agric. Food Chem.* **2002**, 50,1168–1171.
[15] IFF Benzie;JJStrain; *Analytical Biochemistry*.**1996**,239,70-76.
[16] B Bozin;N Mimica-Dukic;I Samojlik;AGoran;RIgic; *Food Chemistry*. **2008**,111, 925–929.
[17] QCai;ORahnh;R Zhanga; *Cancer Letters*. **1997**, 1, 99- 107.

[18] J Kukic;VNPopovic;SPetrovic;PMucaji;ACIric;DStojkovic;M Sokovic; *Food Chemistry*. **2008**, 107, 861–868
[19] JRNguemeving;AGBAzebaze;VKuete;NNECarly;VPBeng;MMeyer;ABlond;BBodo;AENkengfack ; *Phytochemistry*. **2006**, 67, 1341–1346.
[20] JPeng, GFan, YWu; *J. Chromatography A.* **2006**, 1115, 103–111
[21] ABCerezo;ECuevas;PWinterhalter;MCGarcia-Parrilla;AMTroncoso; *Food Chemistry*. **2010**, 123, 574-582.
[22] SEDrewes;SFVVuuren; *Phytochemistry*.**2008**,69,1745-1749.
[23] CCWang;SCChang;BSInbaraj;BHChen; *Food Chemistry*. **2010**, 120, 184-192.
[24] J Du;ZDHe;RW Jiang;WCYe;HXXu;PP But; *Phytochemistry*. **2003**, 62, 1235-1238.
[25] NFang, JE Casida; *J. Nat. Prod.* **1999**, 62, 205–210.
[26] OPSharma;TKBhat; *Food Chemistry*.**2009**, 113, 1202-1205.
[27] MTomczyk;KPeter Latte; *Journal of Ethnopharmacology*. **2009**, 122, 184-204.
[28] TPTCushnie;AJLamb; *International Journal of Antimicrobial.* **2005**, 343–356.
[29] QCai;RORahn;RZhang; *Cancer Letters*. **1997**, 119, 99-107.
[30] ZLu;GNie;PSBelton;H Tang;BZhao; *Neurochemistry International*. **2006**, 48, 263–274.
[31] CA Rice-Evans;NJ Miller;GPaganga; *Free Radical Biology and Medicine*.**1996**,20,933-956.