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Original Article

Inhibition of Oral Squamous Cell Carcinoma by Herbs

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Abstract*: The phytochemical contents of Cyperusrotundus and Commiphora Myrrhawere carried out in a quest to identify their potential as a source of alternative medicine. The qualitative and quantitative analysis has done to find out the maximum yield of secondary metabolite for the aqueous and methonalic extract of Cyperusrotundus and Commiphora Myrrha .Antibacterial activity against five strains of bacteria(Klebsiella,Staphylococcusaureus, pneumoniae, Proteus mirabilis, Enterococcus sp, E.coli) and antioxidant activity were identified for the methanolic extract of Cyperusrotundus and Commiphora Myrrha. The results showed that the extracts of Cyperusrotundus and Commiphora Myrrha could be a scope for exploitation of these phytochemicals in the field of pharmaceutical and medicine industries.*

Keywords*: Methanolicextract,,Cyperusrotundus,Commiphora Myrrha*

INTRODUCTION

Medicinal plants are embodiment of variety of secondary metabolites which require carrying out many important biological functions, to defend against attack from predators such as Pathogens ,insects, fungi and herbivorous mammals. So far, 12,000 chemical compounds isolated and estimated to be less than 10% of total[1] (Tapsell LC,2006).Secondary metabolites in plants regulate the body function of human, as our conventional drugs do. Herbal medicines possess the mechanism identical to already existing conventional drugs. The great advantage of herbal medicine is its devoid of side effect, at same time they do not vary greatly from existing conventional drugs in terms of working mechanism. So they are as great as conventional medicines .[2] (Lai PK, Roy J, 2004).Therefore, screening test is carried out to evaluate their use in folk medicines to find out the active principle by characterization and isolation of constituents. Methodical screening of them leads discovery of novel active compounds.

1. Plant Description
2. Commiphoramyrrha (Balsamodendron Myrrha, Gum Myrrha Tree)

In pharmaceutical, myrrh has been used as an antiseptic in tooth paste , mouthwashes, and gargles, to treat gum disease and also to used as an analgesic in Toothaches, bruises liniment and sprains.[5](Lawless, 2002).***Cyperusrotundus(***Coco Grass, Purple Nut Sedge, Red Nut Sedge, Mustaka, Nut Grass)I Chinese traditional medicinesays that Cyperus is a primary regulating herb and also pronounced the same in Indian Ancient Ayurvedic. In Modern Ayurvedic Cyperus Known to bemusta or mustamoolachurna, to treat fevers, disorders of digestive system, dysmenorrhea and other maladies.[6](Manish, 2010). But our work highlights on herbal treatment of *Cyperusrotundus* and *Commiphoramyrrha*against the Oral Squamous Cell Carcinoma and five clinical pathogen. (i.e)*Klebsiellapneumoniae, Staphylococcus aureus, Proteus mirabilis*, *Enterococcus sp, E.coli*.

MATERIALS AND METHODS

Collection and identification of plant sample:

The plant leaves of Cyperus*rotundus* and *Commiphoramyrrha* were collected from Kollihills of Namakkal district, Tamil Nadu. The identified plant species, leaf materials were dried under shade and then powdered by electrical grinder.The powdered each plant leaf samples were stored in a clean paper bags separately for further studies[10] (Akinyemi, *et al.,* 2000).

Preparation of plant extracts (Aqueous and Metabolic):

Extract of plant aerial part (leaves) was prepared by using two different solvents such as methonal and water. Dried and powdered plant leaf materials used for extract preparation by using soxhelet apparatus. The

extract were evaporated to complete dryness by a vacuum distillation and stored in refrigerator for further use(old ref).

Qualitative Analysis of secondary metabolites:

Plant leaf extract of Cyperusrotundus and Commiphoramyrrha subjected to preliminary screening of secondary metabo lites such asSaponin(14),Alkaloids,Polyphenols(15),Glycosides,Flavanoids,Tannins,Steroids(16-19) following by using the above reported methods with small modification.

Antioxidant activity (DPPH free radical scavenging activity) of the leaf extraction (Aqueous and Methanolic) of *Cyperusrotundus* and *Commiphoramyrrha* .The scavenging activity of plant leaf extract was assessed by using spectrophotometric method to identify the presence of DPPH as a free radical.1ml of plant leaf extract was added into 0.002% DPPH solution.This mixture mixed well and kept incubation for 30 min in dark at the room temperature.The absorbance was measured at 517 nm.

Percent (%) inhibition

DPPH activity = $\frac{A(control) - A(sample)}{A(control)} \times 100$

A control=Absorbance of the control sample

A sample=Absorbance of the sample containing plant extract/ standard.

Antioxidant activity of Cyperusrotundus and Commiphoramyrrha have expressed in terms of IC_{50} values.

Calibration curve for Ascorbic acid (vitamin C) used as standard.()

Antimicrobial activity by Agar well diffusion assay (Methanolic extract of *Cyperusrotundus* and *Commiphoramyrrha)*[16](Perez, *et al.*, 1990)

The agar well diffusion method was used to evaluate the antimicrobial activity of plant material. The dried plant leaf extract of Cyperusrotundus and Commiphoramyrrha were reconstituted with Dimethyl sulfoxide (DMSO) to obtain a stock solution of 200 mg/mL-1, 100 mg/mL-1, 50 mg/mL-1, and 25 mg/mL-1were prepared. Muller Hinton agar plates were prepared and the plates were swabbed using sterile cotton swabs with the adjusted broth culture of the respective bacterial strains. Five wells with 6 mm in diameter were made equidistance in each of the plates using a sterile cork borer. Up to 25µl to 100 µl of each concentration of the extract were respectively introduced into the wells using sterile automatic pipettes, with the stock in one well. It was allowed to diffuse at room temperature for 2 hrs and the plates were incubated to 37°C for 24 hrs. Diameters of the inhibition zones were measured. The antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the plant.

ANTICANCER ACTIVITY – CYTOTOXICITY STUDY

Human cell lines:

HSC-4 (Human oral squamous carcinoma) human cancer cell lines of mouth grown in RPMI medium were obtained from CMC Hospital Vellore

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In-vitro cytotoxicity studiesof the leaves extract (Methanol) of *Cyperusrotundus* and *Commiphoramyrrha*[17](Sini, *et al.,* 2012) Short term in vitro cytotoxicity study was done for the methonolic extract of plant sample by using HSC-4. In an Eppeandorf vial of capacity 1ml,10mg of the extract , diluted to six different concentrations along with its duplicate and control (50%) by using DMSO as a solvent and mixed well with the help of a vortexing machine. Cell viability checked by checked by trypan blue dye (1%). The cell suspension ($1x10^6$ cells in 0.1ml) was added to tubes containing various concentrations of the test compounds and the volume was made up to 1ml using Phosphate Buffered Saline (PBS). Control tube has only cell suspension. These assay mixtures were incubated for 3 hour at 37°C. After incubation 0.1 ml trypan blue was added and found number of dead cells determined by using haemocyto meter. The percent viability was calculated .% viability = (live cell count/total cell count)X 100.

MTT ASSAY (MICRO CULTURE TETRAZOLIUM)

Cell viability was assessed for different concentrations of the plants extractby MTT assay (Micro culture tetrazolium/formazan assay). The cells were feeded in 96-well plates. Four wells for each concentration were seeded and triplicate plates were used the cell line. Then the cells were incubated at 37°C. Every 24 h fresh medium was placed containing different concentrations of extract. After, the medium was changed by fresh medium containing MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 4-diphenyltetrazolium bromide] with a final concentration of 0.5 mg/ml (after 24 h). The cells werekept incubationfor another 4 h in a humidified atmosphere at 37°C then the medium containing MTT was removed and the MTT formazan crystals were dissolved in DMSO. The absorbance measured by ELISA reader at 570nm. $IC_{50}$was the concentration of the extract which was produced a 50% decrease in cell viability to the negative control that was wells exposed to the solvent without extract.IC_{50} is used to relate competitive agonists and antagonists by the Cheng-Prusoff equation, it is not a direct indicator of affinity. For enzymatic reactions, this equation is:

$$K_i = \frac{IC_{50}}{1 + \frac{[S]}{K_m}}$$

where K_i - binding affinity of the inhibitor, IC_{50} - functional strength of the inhibitor, [S] - fixed substrate concentration and K_m - concentration of substrate at which enzyme activity is at half maximal.

RESULT AND DISCUSSION

The plant Cyperus*rotundus* and *Commiphoramyrrha* was collected from Kolli hills of Namakkal district, Tamil Nadu. The leaf materials dried under shade and then powdered with a mechanical grinder. The dried powdered of leaf materials of *Cyperusrotundus* and *Commiphoramyrrha* were extracted with different solvents used successively with gradient polarity Methanol and water in a Soxhlet's apparatus. The extracts were evaporated to complete dryness by vacuum distillation.

Table-1
Phytochemical analysis of Cyperus*rotundus* and *Commiphoramyrrha* Leaf Extract

Phytochemical tests	***Cyperusrotundus***		***Commiphoramyrrha.***	
	M	***A***	**M**	***A***
Tannins	+++	++	+++	++
Saponins	+++	++	++	+
Flavonoids	+++	++	+	+
Steroids	+++	++	+	+
Glycosides	+	+	+	+
Alkaloids	+++	++	++	+
Anthroquinones	+	+	+	+
Phenolic compounds	++	+	++	+

Where the M-methanol extraction, A-Aqueous extraction, the presence of the phytochemicals denoted by the code +++ (Maximum), ++ (Moderate), + (Minimum) Table-1 represents the qualitative analysis of the Phytochemicals of *Cyperusrotundus* and *Commiphoramyrrha***.** The presence of the Tannins, Saponins, Phenolic compounds, Steroids, Glycosides, Alkaloids, Anthroquinones, Flavonoids in methanol extracts *Cyperusrotundus* and *Commiphoramyrrha*.

Table -2
Quantitative Analysis of Cyperusrotundus and Commiphoramyrrha

Phytochemicals	***Cyperusrotundus* (Methanolicextraction)**	***Commiphoramyrrha*. (Methanolicextraction)**

	mg/ml	mg/ml
Phenol	2.345±0.0021	1.000±0.002
Flavonoids	2.456±0.01	0.200±0.01
Tannins	2.200±0.001	0.111±0.002
Saponins	1.231±0.012	0.056±0.01
Carbohydrates	1.456±0.002	0.413±0.01
Proteins	2.854±0.001	0.600±0.001
Amino acids	2.000±0.001	0.112±0.001

Quantitative analysis of Protein, Saponins, Tannins, Flavonoids, Carbohydrate and Phenol, Aminoacids were analysed for the *Cyperusrotundus* and *Commiphoramyrrha* as shown in the Table **-2 .** Each value is represented as mean± S.E. (n=3)

Table -3

Antioxidant Activity of Cyperusrotundus and Commiphoramyrrha.

Assay	***Cyperusrotundus* (Methanolic extract)**	***Commiphoramyrrha*. (Methanolic extract)**
DPPH ASSAY	35%	24%

The antioxidants were analyzed and the Antioxidant activity (DPPH free radical scavenging activity) were shown in the Table -3

ANTIMICROBIAL ACTIVITY

Antimicrobial activity by Agar well diffusion assay:

The different extracts of two plant species possessed antibacterial activity. The antimicrobial activity was assessed against the clinical pathogen, (*Klebsiella_Staphylococcusaureus, pneumoniae, Proteus mirabilis, Enterococcus sp, E.coli*). The highest zone of growth inhibition was shown the by methanolic extract of *Cyperusrotundus* (100 µl) against five clinical pathogen, when compared with *Commiphoramyrrha.*

Table -4

Antimicrobial activity of*Cyperusrotundus* and *Commiphoramyrrha*.

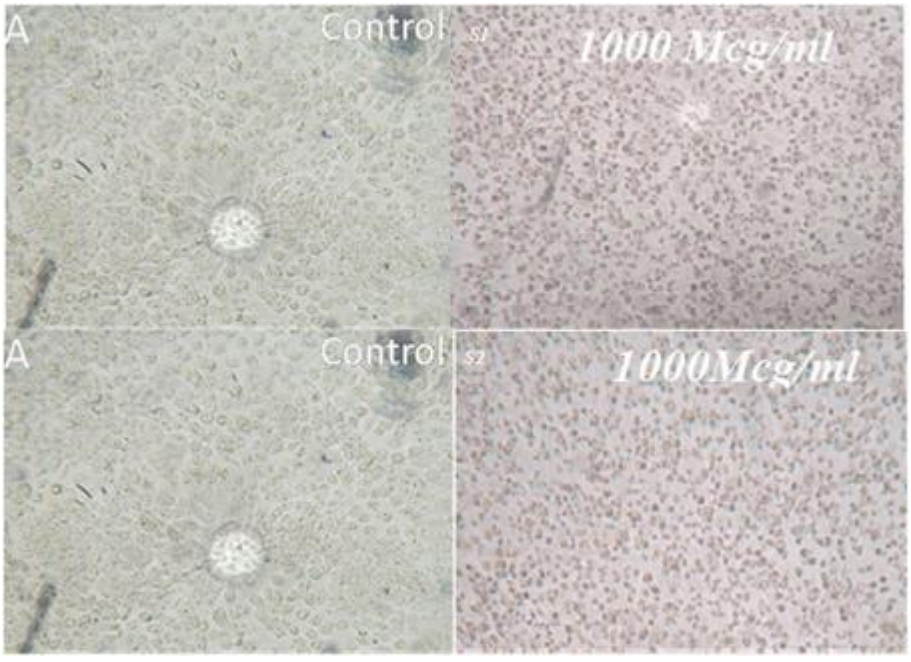
Clinical pathogen	**Clear zone of inhibition(in mm/ml)**	
	***Cyperusrotundus*(Methanolic extract)**	***Commiphoramyrrha*. (Methanolic extract)**
E.coli	18	16
Klebsiellasp	22	18
Staphylococcus aureus	20	16

Enterococcus sp	22	18
Proteus mirabilis	24	16

Table 5
CYTOTOXICITY STUDIES
Cytotoxicity studies of plant sample (methanolic extract):

Plant extract	Concentration Mcg/ml	Absorbance	% of inhibition	Inhibitary Concentration (IC 50) Mcg/ml
***Cyperus rotundus* (S1)**	50	0.428	49.16	128
	125	0.382	54.63	
	250	0.266	68.40	
	500	0.162	80.76	
	1000	0.002	99.80	
***Commiphoramyrrha* (S2)**	50	0.410	51.30	125
	125	0.325	61.40	
	250	0.231	72.56	
	500	0.112	86.69	
	1000	0.002	99.81	

The drug showed nontoxic significant effect at 50, 125, 250, 500, 1000 Mcg/ml concentrations.(Mcg-Micro gram) Cytotoxicity studies of methanolic extract of Cyperus*rotundus and Commiphoramyrrha*



DISCUSSION

The plant *Cyperusrotundus* and *Commiphoramyrrha* was collected from Kolli hills of Namakkaldistrict ,Tamil Nadu. The leaf materials dried under shade and then powdered with a mechanical grinder. The dried powdered of leafs of *Cyperusrotundus* and *Commiphoramyrrha* were extracted with different solvents successively with gradient polarity (Methanol& Aqueous) in a Soxhlet's apparatus. The extracts were evaporated to complete dryness by vacuum distillation.Table1 represents the qualitative analysis of the Phytochemicals of *Cyperusrotundus* and *Commiphoramyrrha*. The presence of the Tannins, Saponins, Phenolic compounds, Steroids, Glycosides, Alkaloids, Anthroquinones, Flavonoids in methanol extracts *Cyperusrotundus* and *Commiphoramyrrha***.** Quantitative analysis of Protein, Carbohydrate and Aminoacids were analysed in *Cyperusrotundus* and *Commiphoramyrrha*.The antimicrobial activity was assessed against the clinical pathogen, (*Klebsiella, taphylococcusaureus*, *pneumonia ,Proteus mirabilis, Enterococcus sp, E.coli*). The highest zone of growth inhibition was shown by methanolic extract (100 µl) against five clinical pathogen, the highest zone of inhibition was noticed in *Cyperusrotundus* leaves when compared with *Commiphoramyrrha***.** This antioxidant potential of *Cyperusrotundus and Commiphoramyrrha* could be attributed to the presence of flavonoids, alkaloids, betacyanins, quinones, terpenoids, and phenols which showed the result of about 34% and 25% .This study examined the cytotoxicity activity of extracts and purified components from *C. myrrha.* The result showed that the activity of about 128 IC 50Mcg/ml and 125 IC 50 Mcg/ml *Cyperusrotundus and Commiphoramyrrha* respectively.

CONCLUSION

The crude methanolic extracts of *Cyperusrotundus and Commiphoramyrrha* showed significant antimicrobial activity against five bacterial strains. The extracts revealed equal or higher broad-spectrum antimicrobial activity. This indicated the great potential of these plant extracts as effective antimicrobial agents that can be used as single or in combination in medicines or can be used as natural food preservatives to retain the quality of food and prevent its spoilage. The extracts were found rich in phytochemical constituents, which are responsible for their potent antimicrobial properties. These individual constituents of the extracts can be isolated, and further characterization as well as quantification can be done to explore the potential of these antimicrobial agents present in the extracts. The results were expressed as the concentrations causing 50% cell growth inhibition. The inhibition of cell proliferation and viability appeared to be highly dose-dependent. The most active *rotundus* species against the HSC-4 cells were *Cyperusrotundus* .So we can use this plant species to *prepare* tooth paste which has the ability to inhibit mouth cancer and mouth bacteria's.

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