Current Trends in Biotechnology and Pharmacy
Vol. 16 (2) 144 - 154, April 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.2.14

In Vitro anti-Protozoan Activity of Methanolic Extracts of *Caralluma procumbens* Against *Tritrichomonas foetus*.

Rajani Vemula[1], Gayathri Pachipala[1], P.V.B Reddy[3], Sathish Kota [4], M. Raman [4], Uday Sankar Allam[1], Kota Neela Manikanta[2] & Kiranmai Chadipiralla[1*]

[1] Department of Biotechnology, Vikrama Simhapuri University, Nellore-524320, Andhra Pradesh, India
[2] Department of Tourism, Vikrama Simhapuri University, Nellore-524320, Andhra Pradesh, India.
[3]Department of Life Science & Bioinformatics, Assam University Diphu Campus, Diphu-782462, Assam
[4] Translational Research Platform for Veterinary Biologicals (TRPVB), Chennai-51
*Corresponding Author: cdpkiranmai@gmail.com

Abstract

Tritrichomonas foetus is a flagellated venereal parasite that causes Trichomoniasis. Among various infections, *Tritrichomonas* infection is a major threat to animal husbandry contributing to heavy economic loss due to fetal deaths or abortions. Plants are a great source of a variety of secondary products that consist of different bioactive compounds with medicinal properties. Species of *Caralluma* are known to be sources of potential therapeutic molecules. However, no studies have been carried out on the anti-protozoan activity *of Caralluma procumbens* against *Tritrichomonas foetus*. Hence the effect of different concentrations of *Caralluma* extract on the growth and survival of *Tritrichomonas foetus* was examined. Methanolic extract of *Caralluma procumbens*, at the concentrations of 5 and 10 mg/ml inhibited the growth of *Tritrichomonas foetus* completely after 24 hours of incubation. A concentration of 2 mg/ml inhibited 80% growth of *Tritrichomonas foetus* after 48 hours of incubation with *Caralluma* extract. The results of the current study suggest that *C. procumbens* could be suitable for treatment and prevention of protozoan induced Trichomoniasis and prove to be an effective therapeutic strategy without any nonspecific effects. Further studies in this line will help in identification of effective structural bioactive components present in the methanol extract of *C. procumbens.*

Keywords: Bioactive Compounds, *Caralluma procumbens*, Methanol Extract, *Tritrichomonas foetus, Trichomoniasis.*

Introduction

Tritrichomonas foetus is a single cell flagellate parasite, known for causing infections in the reproductive tracts of bovine and intestinal tract of cats (1). *Tritrichomonas* belongs to the kingdom Protoctista. These are spindle shaped flagellate parasites with their size ranging from 5 to 25µm. *T. foetus* consists of three anterior flagella, one posterior flagellum with an undulating membrane (2). It is also known as venereal pathogen of the cattle that spreads through sexual intercourse. It has been recognized as major threat in the cat families specifically in the domesticated ones. This protozoan is an enteric pathogen usually residing in the inner lining of the colon (3). *Ttitrichomonas foetus* is also known to cause Feline Trichomonias, a large-bowel disease in cats (4,5). *T. foetus* has been reported to induce spontaneous abortions in the first trimester of pregnancy in cats. Further it is also known to be a causative factor responsible for infertility (1, 6).

In addition to cat, this pathogen is also known to infect cattle. Symptoms of *T. foetus*

infection are usually not expressed by bulls however can infect females after mating. These parasites are usually found in abundance in the vagina and cervix of the cows (7). The pathogenic protozoan-induced abortion has been recognized as one of the most significant cause of bovine abortions (8). Among the various infections, the *Tritrichomonas* infection has been a major threat to the animal husbandry and cattle industry contributing to major economic loss every year due to fetal death or abortions.

The common drug used to manage the infected cats effectively against this flagellate is Ronidazole. A concentration of 30 to 50 mg/kg bodyweight inhibits the growth of *T. foetus* parasites. Bovine Trichomoniasis can also be treated by using Dimetridazole at a high concentration of 50mg/kg bodyweight per day for 5 days orally (9). Although these are being used for management of the disease and encountering the pathogenic effects of this protozoan, unfortunately, it is reported that this drug induces certain side effects eventually resulting in neurotoxicity in some cats along with associated symptoms that may include lethargy, cutaria, seizures etc (10). In order to address these issues related to the existing drugs, there is a need for the development of alternate methods of treatment of *T. foetus*.

Plants have always been regarded as a great source of a variety of secondary products that consist of different types of bioactive compounds with medicinal properties. Since plants are enriched with such products, they have gained importance in the field of therapeutics and drug designing protocols that are proving to be more efficient and yet safer and available at cheaper cost making them available to a wider range of population.

The genus *Caralluma* consists of around 120 species of flowering plants belonging to family Apocynaceae. *Caralluma* plants are succulent perennial herbs that are edible. Many of the *Caralluma* species are found to possess high medicinal properties. Earlier studies have reported that *Caralluma* species possess high medicinal value and hence found place in traditional and folk medicine (44, 45). Several studies have identified the phytotherapeutic properties associated with the plant extracts of various *Caralluma* species (46-48). In traditional system of medication, *Caralluma procumbens* has been reported to be effectively rectifying the bowel complaints and the oil extracted from this plant are used to cure rheumatism (11). The Antimicrobial activity of *C. procumbens* has been proved and showed its pharmacological importance (11). This plant can be used as antimicrobial agent.

As said earlier the *T. foetus* infection has been a major threat to the animal husbandry in general and cattle in particular. There is an urgent need for identifying and developing novel methods of encountering the damage caused by the lethal flagellate. Further it is also reported that the current drugs could cause certain side effects to the cattle. Keeping these points in focus, there is a need to identify nontoxic, safer and cheaper compounds that could inhibit the growth and survival of the infection causing *T. foetus*. The objective of our study is to determine the protective effect of the extracts of the succulent, *C. procumbens* against *T. foetus.*

Materials and Methods

The plant *Caralluma procumbens* was collected from rocky hills of Maruthuvamalai hills of Kanyakumari district, Tamilnadu. The plant was identified and authenticated by a plant taxonomist. The stems of *C. procumbens* were collected and washed under running tap water to remove dust particles. Following the wash, the stems were rinsed with double distilled water. The stems were then chopped into small pieces, shade dried and thoroughly ground to make fine powder. This powder was subjected to soxhlet and collected the extract using rotary evaporator and stored in a refrigerator until further usage. Methanol extract was prepared by boiling 5 gm of plant powder in 100 ml of methanol separately in screw cap bottle and placing in water bath for

two hours. The temperature was maintained at 80°C.The extract was filtered using whatman filter paper (No.1) and then subjected to rotary evaporator and collected the extract.

Qualitative phytochemical analysis

The concentrated extracts prepared from *C. procumbens* were used for preliminary phytochemical screening and estimation of secondary metabolites. The concentrated extracts ware dissolved again in methanol and used for screening. The following tests were conducted for screening of the phytocompounds.

Test for tannins

Ferric chloride test: The test for tannins was performed following the method of Iyengar (12). To the 2 ml of already prepared plant extract, 2 ml of 5% ferric chloride was added in a boiling tube. Formation of a dark blue or greenish black colour reveals the presence of tannins.

Test for saponins

Foam test: The test for the presence of Saponins was performed according to the method of Kokate Kokate (13). To estimate the Saponins, 2 ml of already prepared plant extract was mixed with equal volume of distilled water. The mixture in the tube was gently shaken for 15 minutes. Appearance of foaming layer reveals the presence of saponins in the extract.

Test for flavonoids

Test for the presence of flavonoids was performed following the method of Gowri and Vasantha (14). Plant extract (2ml) was pipetted into a boiling tube to which 5 to 10 drops of diluted HCl and small amount of Zn was added and was boiled for few minutes. Appearance of reddish pink or dirty brown colour demonstrated the presence of flavonoids in the sample.

Test for alkaloids

Plant extract was tested for the presence of Alkoloids by Mayer's test following the protocol as described by Harborne (15). To a 2 ml of plant extract, an equal volume of concentrated hydrochloric acid was added in a test tube. Following this, a few drops of Mayer's reagent (Potassium Iodide + Mercuric Chloride) was slowly added. The appearance of green color or white precipitate indicates the presence of alkaloids.

Test for quinines

The presence of quinines in the plant extract was performed according the method of Trease and Evans (16). To a 2ml of plant extract, another 2 ml of concentrated Sulphuric acid was added. Formation of red color in the solution indicates presence of quinines.

Test for glycosides

Glycosides in the extract of *C.procumbens* were performed by adapting the method of Das and Bhattacharjee (17). To a 2 ml of plant extract, 3 ml of chloroform and 10% ammonia solution was gradually added along the walls of test tube. A change of colour to pink indicates presence of glycosides.

Test for terpenoids

Plant extracts were tested for the presence of Terpenoids according to the method of Siddiqui *et al* (18) To a 2 ml of extract, 2 ml of chloroform was added and concentrated Sulphuric acid was added carefully in a test tube. Appearance of reddish brown color layer indicates the presence of terpenoids.

Test for phenols

Presence of Phenols in the plant extract was performed according to the method described by Wolfe *et al* (19). To a 2 ml of the *Caralluma* extract, 2 ml Folin-Ciocalteu reagent and 4 ml of sodium carbonate were added in a tube. The mixture was incubated at 40°C for 30 min. Appearance of blue colour indicates presence of phenols.

Test for Coumarins

Current Trends in Biotechnology and Pharmacy
Vol. 16 (2) 144 - 154, April 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.2.14

Coumarins in the extracts of *C. procumbens* were tested for their presence as per the method of Bruneton (20). To a 2 ml of plant extract, 2 ml of 10% sodium hydroxide was added. Formation of yellow color indicates presence of coumarins.

Test for phytosteroids

The test for the presence of phytosteroids was performed as per the protocol of Trease and Evans (21). 2 ml of the plant extract was slowly mixed with equal volume of chloroform in a tube followed by the addition of 2-3 drops of concentrated Sulphuric acid slowly. Appearance of a bluish brown ring indicates the presence of phytosteroids.

Test for phlobatannins

The presence of phlobatannins in the plant extract was tested employing the Hydrochloric acid test following the protocol as described by Brain and Turner[22]. To a 2 ml of plant extract few drops of 2% Hydrochloric acid was gently added in a test tube. The formation of a red colour precipitate in the mixture revealed the presence of phlobatannins.

Test for anthraquinones

Ammonia test as described by Sofowora (23) was used for testing the presence of Anthraquinones. 2 ml of plant extract was pipetted into a tube and few drops of 10% Ammonia solution were added to it. Formation of pink coloured precipitate indicated the presence of anthraquinones.

Culturing of *T. foetus*

The protozoan, *Tritrichomonas foetus (Riedmuller) Wenrich and Emerson (ATCC®30003TM)* was obtained from American Type Culture Collection (ATCC) by Translational Research Platform for Veterinary Biologicals (TRPVB), an affiliated unit of Tamil Nadu Veterinary and Animal Sciences University (TANUVAS). Growth conditions were optimized at TRPVB and the culture along with sufficient numbers of Inpouch TF kits (BIOMED, USA) were kindly provided to our laboratory. *T. Foetus* were inoculated in 10 ml of sterile diamonds modified medium and allowed to grow for 24h at the temperature of 36°C. The cell growth was observed under compound microscope after 24h. The cells were counted and noted The cells were seeded at a density of $1x10^6$cells/ml. Motility and activity of cells was observed under microscope.

Preparation of Sterile Diamond Modified medium: The following ingredients were used for the preparation of 1litre of Diamond modified medium such 30 g Yeast, 0.5g Maltose, 1.0gm L-Cysteine Hcl, 0.2 g L-.Ascorbic acid, 0.8 g K_2HPO_4, 0.8 g KH_2PO_4, 0.5g Agar Noble. All these ingredients except Agar were mixed in distilled water and the pH was adjusted to 7.2. Following the adjustment of pH, Agar was added to the medium and was sterilized by using autoclave. This sterilized media was used for culturing and multiplication of *T. foetus.*

Effect of different concentrations of plant extracts on *tritrichomonas foetus*

The current study is an experimental and observational study. Different concentrations of methanolic plant extracts in the order of 0.5, 1, 2, 5 and 10 mg/ml were prepared. Methanol was used as control. At the exponential phase, about 10 µls of *T. foetus* culture was transferred to InPouchTM TF pouches along with a supplementation of a plant extract at a defined concentration. Here we used InPouchTM TF pouches to reduce contamination and convenience for observation under phase contrast microscope. Following the treatment with the different concentrations of *Caralluma* extracts, these pouches were incubated at 36°C. After the supplementation of the methanolic *Caralluma* plant extract the pouches containing the cultures were observed for the survival of *T. foetus* using phase contrast microscope at regular intervals of 0.5, 2, 8, 24, 48 and 72 hours. The experiment was performed in three sets with two replicates in each set.

Preparation of Drug (Positive Control):

The drug metronidazole was purchased and was diluted in saline to prepare stock solution. From this stock solution, the drug was added to commercially available InPouchTM TF pouches, containing 10 ml of culture media to get the final concentration of 0.5 µg/ml. This drug containing pouch was used as positive control group.

Results and Discussion

In this current study, the methanol extracts of *Caralluma procumbens* were tested to determine the antiprotozoan activity. Various doses of methanolic extracts of *C. procumbens* were tested for their inhibitory activity on *Tritrichomonas foetus.* Medicinal properties of the plants are determined by their phytochemical & other chemical constituents. The presence of several chemical components has been demonstrated by number of phytochemical studies. These qualitative studies of phytochemicals will give the presence or absence of Secondary metabolites like tannins, flavonoids, alkaloids, quinines, glycosides, terpenoides, phenols, coumarins, phytosteroides, phlobatanines, anthraquinones and saponines etc. In order to understand the composition and various bioactive compounds in the methanol extract of *C. procumbens* were estimated using biochemical assays. The presence of various compounds in *Caralluma* extract were determined and presented in Table I.

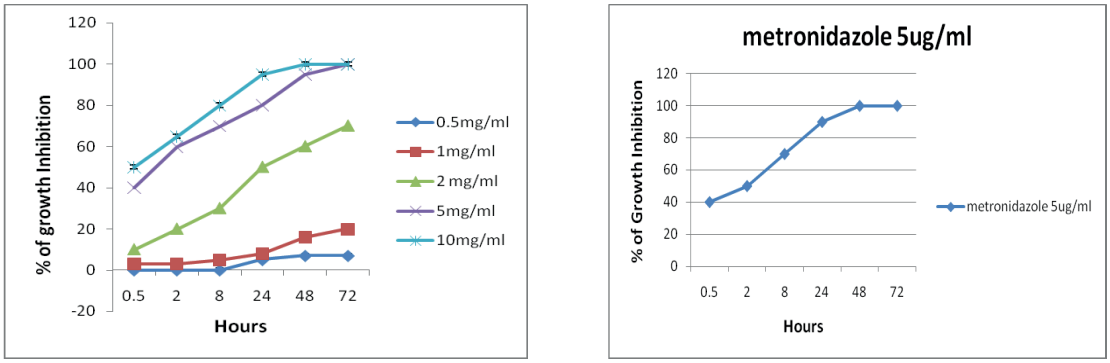
Table 1: Phytochemical components present in the methanol extract of the stems of *C. procumbens*

S.No	Phytochemicals	Activity
1	Tannins	+++
2	Flavonoids	+
3	Alkaloids	+++
4	Quinines	++
5	Glycosides	+++
6	Terpenoids	++
7	Phenols	+++
8	Coumarins	++
9	Phytosteroids	-
10	Phlobatannins	-
11	Anthraquinones	-
12	Saponins	++

"+" *indicates low activity*, "++" *indicates medium activity*, "+++" *indicates high activity*

Caralluma inhibited the growth and multiplication of *T. foetus* in dose dependent manner. The response of the protozoa following treatment with plant extract were observed at regular time intervals of 0.5h, 1h, 2h, 8h, 24h, 48h and 72h. The extract at a concentration of 5mg/ml and 10mg/ml effectively inhibited the growth of *Tritrichomonas* protozoan by 24h. Although the inhibitory action of the plant extract had started as soon as 0.5h, such inhibition gradually increased and peaked by the end of 24h. Following the treatment at a lower concentration (0.5mg/ml, 1mg/ml) of methanolic *Caralluma* extracts, there was no inhibitory effect even after 8h incubation. Treatment of protozoan cultures with plant extract at a concentration of 2mg/ml resulted in activation and reduced movement of the protozoa by the end of 2h. Further a prolonged incubation resulted in a significantly higher level of inhibition of *T. foetus* growth. Overall, the results indicate that the best inhibition of the growth of the *T. foetus* was found at 5mg/ml and 10mg/ml when incubated for 24h (Fig# 1a).

In order to understand and validate the effects of the plant extracts on the *T. foetus*, the effect of a positive control, Metronidazole was also examined in parallel sister culture pouches (Fig#1b). Further, methanol only treatments were used as a control to eliminate the non specific effects of the vehicle if any. Our study found no such effects following methanol treatments alo ne.



(a) **(b)**

Fig 1: Percentage of growth inhibition of *T. foetus* exhibited by different doses of Methanol extract of *C. procumbens* and Metranidzole**.**

Tritrichomonas foetus, a flagellate protozoan is a well recognized venereal pathogen of the cattle. It has also been demonstrated to be present in the inner linings of the colon resulting in diarrhea (1, 4). While on one hand the mechanism of infection is not clearly understood, and on the other hand the rate of infections have been on the rise and is known to be globally wide spread infection. In cattle the infection with the *T. foetus* causes Bovine *Trichomoniasis* and is a sexually transmitted disease. The parasite also results in fetal abortions further leading to infertility problems on long term (1, 6). As of today there is no efficient and complete treatment against the *T. foetus* infection. However the only drug commonly used for the management and treatment of this disease is Ronidazole and Metronidazole. While the use of this drug does not cure the infection completely, it is reported to cause side effects (24). Hence there is an urgent need for identifying an alternative and effective drug with less side effects are necessary.

Since ancient times people have been using medicinal plants for the treatment of various diseases due to the presence of various phytochemicals such as alkaloids, glycosides, tannins, phenolic compounds etc. Plants have been used in traditional and folk medicine to cure several infections and parasitic diseases. Phytotherapy has been greatly exploited in search of novel alternative therapeutic compounds and plays a key role in manifestation of pathogenic mechanisms in various diseases.

The genus *Caralluma* belonging to the family Apocynaceae, has been identified to possess high therapeutic potential and has been in use in the folk and traditional medicine. Many of the species of *Caralluma* are edible and are known to be good supplements of health improving nutrients. Various species of *Caralluma* have been used for the treatment of many diseases like rheumatism, diabetes, leprosy and as antiseptics and as disinfectants (25).

Although *Carallumas* have been widely sought after in the therapeutics, no studies have been focused on understanding the effects of *Carallumas* on the antiprotozoal activity against *T.foetus.* Our study is the first of its kind to demonstrate the protective effects of *Caralluma procumbens* on the venereal protozoan parasite *T.foetus.* The results indicates that the pretreatment of the *T.foetus* cultures with 5 and 10 mg/ml of methanolic extracts of *C. procumbens* attenuated the multiplication and growth of the disease causing protozoa. Further our findings in case of methanolic extracts were in consistence with the drug metranidazole that was used as a positive control. Although this drug is not the best of the treatments against

the *T. foetus* infection, it is under use due to the lack of any other alternative and effective biomolecules. The results of our study could help in directing the research studies to identify the exact bioactive molecules that are executing the antiprotozoal effect. Hence future studies are warranted to isolate and purify the individual biomolecules and determining the biological activity.

Carallumas are rich in several phytochemicals secondary metabolites like tannins, flavonoids, alkaloids, quinines, glycosides, terpenoides, phenols, coumarins, phytosteroides, phlobatanines, anthraquinones and saponines, phenolic compounds (26). Our study demonstrates the presence of various metabolites in the methanolic extracts of *C. procumbens*. Earlier studies have shown that several active substances such as pregnane glycosides, flavonoids and other secondary products have been isolated from various members of *Caralluma* species. Pregnane glycosides including Carambelloside I and II, were shown to be isolated from *C. umbellate* and exhibited increased biological activity (25). The plant extracts of *Caralluma negevensis* yielded around twenty novel pregnane glycosides (27). Similarly, caretroside A and biocide, another kind of pregnane ester glycosides were identified in the phyto extracts of *C. retrospiciens* (28). Pregnane glycosides have also been found in *Caralluma dalzielli* (28), *Caralluma russeliana* (25), *Caralluma negevensis* (29), *Caralluma lasiantha* (30), *Caralluma retrospiciens* (31). Similarly, Oxypregnane glycosides are present in *Caralluma penicillata* (32, 33). In addition to these, other types such as oxypregnane glycosides, Megastimane glycosides have also been successfully isolated from *C. Paniculata* and *C. retrospiciens* respectively(1, 29, 30). Owing to the presence of such diversified therapeutic phytoconstituents, *Carallumas* have been a greatly used in treating various diseases related to inflammation, ulcers, free radical, microbial and parasite mediated disorders.

Although no studies have directly demonstrated the inhibitory activity of *Trichomonas foetus* either by *Caralluma* species or other medicinal plants, few plants have been shown to inhibit the proliferation in other related protozoans. Studies of Abdul Sattar et al have shown that *Caralluma* species are enriched with high levels of pregnane glycosides such as penicilloside E, Caratuberside C that effectively inhibited trypanosomal activity (34). Consistent with these studies our results showed very high inhibitory activity on the growth of a flagellate protozoan *T. foetus* in a dose and time dependent manner. Such inhibitory activity in our study may be attributed to various pregnane glycosides present in *Caralluma procumbens*.

Several phytochemical studies have also demonstrated the inhibitory effects of various medicinal plant extracts on a wide range of other protozoans and microbes. Studies using the methanol extracts of *Ferrula szowipsiana* demonstrated inhibitory effect on the growth and multiplication on trophozoite of *Trichomonas veginalis* (35). This study demonstrated that varied concentrations of methanol extract effectively inhibited 42-68% within 48 h of incubation. Our results were in consistence with their findings showing a dose dependence inhibitory action on the *Trichomonas* protozoa. Similar to our study they demonstrated asignificant reduction in the live parasite in vitro. Effects of several other plants have been shown to be modulators of drug resistance in microbes such as *E.coli* and *Staphylococcus aureus* (36). In vitro protozoan culture studies using essential oils of Sagebrush, Juniper effectively reduced growth of *Trichomanas vaginalis* within 4 hrs (37). Similarly other essential oils extracted from various sources were also found to reduce the growth of *Trichomonas* protozoa. Studies by Yildiz et al showed that essential oils obtained from Zingiber officianale reduced the cell count in different protozoans (38). Eucalyptus extracts prepared in ethyl acetate significantly reduced the protozoan growth and such antiprotozoal activity of plant extract was attributed to the

secondary metabolite compounds present in the extract (39-41). Puk & Guz investigated the therapeutic potential of various plant extracts and found that chest nut, garlic, horse raddish, oregano effectively reduced the growth of *Spironucleus vortens*, a flagellate protozoan parasite found in fish (43). Studies of Camacho-Corona *et al* screened a wide range of Mexican medicinal plants and found that plants such as *Hyptis albida, Smilax app, Crataegus mexicana* exhibited high antiprotozoal activity against gram positive strains such as *Staphylococcus aureus* and *Enterococcus faecalis*. Plant extracts isolated from *Ocimum basilicum, Larrea tridentate, Hyptis albida, Crataegus mexicana* showed high inhibitory activity against *Trichomonas vaginalis, Entamoeba histolitica* (44).

Conclusion

Keeping in view of the huge economic loss to the animal husbandry due to the *T. foetus* infections, the study is highly useful in designing alternate methods of treatment using novel herbal therapeutic molecules, Owing to the vast undocumented ethnobotanical resources with high medicinal properties available in India, only a very minor percentage of plants have been investigated for their phytotherapeutic properties and pharmacological importance. Hence, it is logical to assume that many more useful drugs are present and will be found in the plant kingdom if these entities are searched logically and systematically. Therefore, it is hoped that with further studies and the discovery of active compounds in the species examined and their impact in vivo, a suitable drug can be found for the treatment or recovery of infected cattle with Trichomoniasis.

References:

1. Gookin, J.L., Stebbins, M.E., Hunt, E., Burlone, K., Fulton, M., Hochel, R. and Levy, M. G. (2004). Prevalence of and risk factors for feline *Tritrichomonas foetus* and Giardia infection. Journal of clinical microbiology, 42(6): 2707-2710.
2. Taylor, M.A., Marshall, R.N. and Stack, M. (1994). Morphological differentiation of *Tritrichomonas foetus* from other protozoa of the bovine reproductive tract. British Veterinary Journal, 150(1): 73-80.
3. Gookin, J. L., Breitschwerdt, E.B., Levy, M.G., Gager, R.B. and Benrud, J.G. (1999). Diarrhea associated with trichomonosis in cats. Journal of the American Veterinary Medical Association, 215(10): 1450-1454.
4. Foster, D. M., Gookin, J. L., Poore, M.F., Stebbins, M.E. and Levy, M.G. (2004). Outcome of cats with diarrhea and *Tritrichomonas foetus* infection. Journal of the American Veterinary Medical Association, 225(6): 888-892.
5. Stockdale, H.D., Givens, M.D., Dykstra, C.C. and Blagburn, B.L. (2009). *Tritrichomonas foetus* infections in surveyed pet cats. Veterinary parasitology, 160(1-2): 13-17.
6. Rhyan, J.C., Stackhouse, L.L. and Quinn, W.J. (1988). Fetal and placental lesions in bovine abortion due to Tritrichomonas foetus. Veterinary pathology, 25(5): 350-355.
7. Parsonson, I.M., Clark, B.L. and Dufty, J.H. (1976). Early pathogenesis and pathology of *Tritrichomonas foetus* infection in virgin heifers. Journal of comparative pathology, 86(1): 59-66.
8. Barr, B.C. and Anderson, M.L. (1993). Infectious diseases causing bovine abortion and fetal loss. Veterinary Clinics of North America: Food Animal Practice, 9(2): 343-368.
9. Kimsey, P.B., Darien, B.J., Kendrick, J.W. and Franti, C.E. (1980). Bovine trichomoniasis: diagnosis and treatment. Journal of the American Veterinary Medical Association, 177(7): 616-619.

10. Gookin, J.L., Copple, C.N., Papich, M.G., Poore, M.F., Stauffer, S.H., Birkenheuer, A J. ... and Levy, M.G. (2006). Efficacy of Ronidazole for treatment of feline *Tritrichomonas foetus* infection. Journal of veterinary internal medicine, 20(3): 536-543.

11. Prakash, J.D.P. (2017). Green Synthesis of Silver Nanoparticles by *Boucerosia procumbens* (Gravely & Mayur.). International Journal of Applied Sciences and Biotechnology, 5(2): 168-171.

12. Iyengar, M.A. (1995). (8th edition) Study of Crude Drugs. Manipal.

13. Kokate, C.K. (1999). Practical Pharmacognosy. Vallabh Prakashan Publication. New Delhi, India, 115.

14. Gowri, S.S. and Vasantha, K. (2010). Phytochemical screening and antibacterial activity of *Syzygium cumini* (L.)(Myrtaceae) leaves extracts. Int J Pharm Tech Res, 2(2): 1569-1573.

15. Herborne, J.B. (1973). Phytochemical methods. A guide to modern techniques of plant analysis, 5-11.

16. Trease, G.E. and Evans, W.C. (1989). (11th edition), Pharmacognosy, brailliere tindall.

17. Das, A.K. and BHATTACH. AK. (1970). A systematic approach to phytochemical screening. Tropical Science, 12(1): 54.

18. Siddiqui, A.A. and Ali, M. (1997). Practical pharmaceutical chemistry. CBS Publishers & Distributors.

19. Wolfe, K., Wu, X. and Liu, R.H. (2003). Antioxidant activity of apple peels. Journal of agricultural and food chemistry, 51(3): 609-614.

20. Bruneton, J. Alkaloids. (1999). (2nd edition), Pharmacognosy: phytochemistry and medicinal plants. Paris: Lavoisier publishing 217-20.

21. Trease, G.E. and Evans, W.C. (1983). Text book of Pharmacognosy London. Bailliare Tindall, 12(193): 336.

22. Brain, K.R. and Turner, T.D. (1975). The practical evaluation of phytopharmaceuticals (Vol. 1). Bristol: Wright-Scientechnica

23. Sofowora, A. (1982). Medicinal plants and Traditional Medicine in Africa John Wiley and Sons Ltd. Chichester, England 142-145.

24. Love, D., Fajt, V.R., Hairgrove, T., Jones, M. and Thompson, J.A. (2017). Metronidazole for the treatment of *Tritrichomonas foetus* in bulls. BMC veterinary research, 13(1): 107.

25. Abdul-Aziz Al-Yahya, M., Abdel-Sattar, E. and Guittet, E. (2000). Pregnane glycosides from *Caralluma russeliana.* Journal of natural products, 63(10): 1451-1453.

26. Priya, D., Rajaram, K. and Suresh Kumar, P. (2011). Phytochemical studies and GC-MS analysis of *Caralluma fimbriata* wall. International journal of Pharmaceutical Research and development, 3(10): 105-110.

27. Ramesh, M., Rao, Y.N., Rao, A.A., Prabhakar, M.C., Rao, C.S., Muralidhar, N. and Reddy, B.M. (1998). Antinociceptive and anti-inflammatory activity of a flavonoid isolated from *Caralluma attenuata*. Journal of ethnopharmacology, 62(1): 63-66.

28. De Leo, M., De Tommasi, N., Sanogo, R., Autore, G., Marzocco, S., Pizza, C. and Braca, A. (2005). New pregnane glycosides from *Caralluma dalzielii*. Steroids, 70(9): 573-585.

29. Bader, A., Braca, A., De Tommasi, N. and Morelli, I. (2003). Further constituents from *Caralluma negevensis.* Phytochemistry, 62(8): 1277-1281.

30. Qiu, S.X., Cordell, G.A., Kumar, B.R., Rao, Y.N., Ramesh, M., Kokate, C. and Rao, A.V.N.A. (1999). Bisdesmosidic pregnane glycosides from *Caralluma lasiantha*. Phytochemistry, 50(3): 485-491.

31. Halim, A.F. and Khalil, A.T. (1996). Pregnane glycosides from *Caralluma retrospiciens*. Phytochemistry, 42(4): 1135-1139.

32. Abdel-Sattar, E., Al-Yahya, M.A.A., Nakamura, N. and Hattori, M. (2001). Penicillosides A–C, C-15 oxypregnane glycosides from *Caralluma penicillata*. Phytochemistry, 57(8): 1213-1217.

33. Abdel-Sattar, E., Meselhy, M.R. and Al-Yahya, M.A.A. (2002). New oxypregnane glycosides from *Caralluma penicillata*. Planta medica, 68(05): 430-434.

34. Abdel-Sattar, E., Harraz, F.M., Al-Ansari, S.M.A., El-Mekkawy, S., Ichino, C., Kiyohara, H. and Yamada, H. (2008). Acylated pregnane glycosides from *Caralluma tuberculata* and their antiparasitic activity. *Phytochemistry*, *69*(11): 2180-2186.

35. Khanmohammadi, M., Ganji, S. and REYHANI, R.S. (2014). Anti-protozoan Effects of Methanol Extracts of the *Ferula szowitsiana* on the *Trichomonas Vaginalis* Trophozoites in vitro. International Journal of Women's Health and Reproduction Sciences, 2(5): 301-306.

36. Fazli, BB, Rezaei, A., Iranshahi, M., Naderi, NM., & Khajeh, KAM. (2009). Evaluating the potentiating effect of galbanic acid from *Ferula szowitsiana* on three common antibiotics against resistant hospital isolates of *Staphylococcus aureus*. Iranian Journal of Pharmaceutical Research, 8(3): 217-221.

37. Petrin, D., Delgaty, K., Bhatt, R. and Garber, G. (1998). Clinical and microbiological aspects of *Trichomonas vaginalis.* Clinical microbiology reviews, 11(2): 300-317.

38. Yildiz, G., Tekeli, A., Drochner, W. and Steingass, H. (2015). Determination of the effects of some plant extracts on rumen fermentation and protozoal counts by hohenheim" in vitro" gas production Technique. International Journal of Animal and Veterinary Advances, 7(2): 18-26.

39. Boussaada, A., Arhab, R., Calabrò, S., Grazioli, R., Ferrara, M., Musco, N. and Cutrignelli, M.I. (2018). Effect of *Eucalyptus globulus* leaves extracts on in vitro rumen fermentation, methanogenesis, degradability and protozoa population. Annals of animal science, 18(3): 753-767.

40. Kumar, R., Kamra, D.N., Agarwal, N., & Chaudhary, L.C. (2009). Effect of eucalyptus (*Eucalyptus globulus*) oil on in vitro methanogenesis and fermentation of feed with buffalo rumen liquor. Animal Nutrition and Feed Technology, 9(2): 237-243.

41. Patra, A.K. and Yu, Z. (2012). Effects of essential oils on methane production and fermentation by, and abundance and diversity of, rumen microbial populations. Appl. Environ. Microbiol., 78(12): 4271-4280.

42. Puk, K. and Guz, L. (2014). Effects of medical plant extracts on the growth of the fish parasite *Spironucleus vortens*. Medycyna Weterynaryjna, 70(3): 165-168.

43. Camacho-Corona, M.D.R., García, A., Mata-Cárdenas, B.D., Garza-González, E., Ibarra-Alvarado, C., Rojas-Molina, A. and Gutiérrez, S.P. (2015). Screening for antibacterial and antiprotozoal activities of crude extracts derived from Mexican medicinal plants. African Journal of Traditional, Complementary and Alternative Medicines, 12(3): 104-112.

44. Malladi, S., Ratnakaram, V.N., Suresh

Babu, K. and Pullaiah, T. (2017). Evaluation of in vitro antibacterial activity of *Caralluma lasiantha* for scientific validation of Indian traditional medicine. Cogent Chemistry, 3(1): 1374821.

45. Rauf, A., Jan, M.R., Rehman, W.U. and Muhammad, N. (2013). Phytochemical, phytotoxic and antioxidant profile of *Caralluma tuberculata* NE Brown. Wudpecker Journal of Pharmacy and Pharmacology, 2(2): 21-25.

46. Tatiya, A.U., Kulkarni, A.S., Surana, S.J. and Bari, N.D. (2010). Antioxidant and hypolipidemic effect of *Caralluma adscendens* Roxb. in alloxanized diabetic rats. Int J Pharm, 6(4): 400-6.

47. Priya, D., Rajaram, K. and Sureshkumar, P. (2014). Evaluation of anticancer activity of methanolic extract of *Caralluma fimbriata* wall against lung cancer cell line. World Journal of Pharmacy and Pharmaceutical Sciences, 3(4): 1263-1271.

48. Al-Faifi, Z.I., Zarraq, A.I., Masrahi, Y.S. and Aly, M.S. (2016). In vitro anticancer activity of *Caralluma acutangula* (Decne.) NE Br. Extract. International Journal of Pharmaceutical Sciences Review and Research, 38(2): 59-63.