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PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *CISSAMPELOS PAREIRA LIN*. RHIZOME EXTRACT AGAINST SOME BACTERIAL STRAIN

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ABSTRACT

The plant *Cissampelos pareira* belonging to Menispermaceae family contains various phytoconstituents with specific value in ethnomedicinal practices which is significant in the field of health care system. The study had been envisioned to evaluate phytochemical and antimicrobial activity of plant's rhizomes with aim to analyze the potentiality of this plant as new potent antibacterial agents. For phytochemical screening; Mayer's reagent test, Baljet reagent test, Gelatin test, Spot test, etc were used and analyzed. In our research, the methanolic and ethyl acetate extract was prepared using the plant's rhizomes by double maceration extraction method and their antimicrobial activity was assessed against *Staphylococcus aureus* & *Escherichia coli* organisms using the standard antimicrobial agents such as Ciprofloxacin & Ampicillin by disc diffusion method. Based on phytochemical screening, the plant extracts contain alkaloids, glycoside, fixed oil, carbohydrate, etc. The methanolic extract showed the highest yield value (8.5552%) in comparison to ethyl acetate extract (3.2872%). The methanolic extracts showed more positive tests for presence of phytoconstituents. Similarly, after analyzing the Zone of inhibition, it was found that Ethyl Acetate extract was more sensitive against S. aureus in comparison to methanolic extract whereas the methanolic extract in turn was more sensitive against E. coli than Ethyl acetate extract. Following the broth dilution method, the minimum inhibitory concentration of methanolic extracts against S. aureus & E. coli was 1.25 mg/ml & 0.56 mg/ml and of ethyl acetate extracts were 0.62 mg/ml & 1.25 mg/ml. The study of various

bioactive substances from our research work recommends its potential use in formulation of antimicrobial agents for the treatment of many ailments.

KEYWORD'S: *Cissampelos pareira*, Phytochemical screening, Antimicrobial activity, Disc diffusion method, Zone of inhibition.

1. INTRODUCTION

Plants and plant- based medication are the basis of many of modern pharmaceuticals we use today for various ailments. Plant-derived substances have recently become of great interest owing to their versatile applications therefore the medicinal value of plants are inherent in some chemical constituents which produce a definite physiological action in human body. The most important of these bioactive constituents of plant are alkaloids, tannins, flavonoids, phenolic compounds, etc. For discovery and also development of novel drugs, various scientists are looking forward to the alternative sources and to develop the new lead molecules for pharmaceutical uses.[1]

The plant *Cissampelos pareira* L. (Fig.1) commonly known as venivel belongs to the family Menispermaceae. The plant is found throughout tropical and sub-tropical region of Nepal, in India from Sind and Punjab to South India and Ceylon. It is a very variable, lofty, slender, dioecious, perennial climber, ascending up to an altitude of 2000 m.[2] The plant flowers during the rainy season as well as autumn and fruits during winter. Seeds are of horse shoe shaped. Roots and rhizomes (Fig.2) are cylindrical, often tortuous light brown to yellowish in color of root but rhizome is dark brownish color. Numbers of species are available throughout the world but only one species is available in India, Nepal and other tropical and sub-tropical country. The roots are used as astringent, antispasmodic, analgesic, diuretic, antilithic and emmenagogue. It is prescribed for diarrhea, dysentery, piles urogenital infections. Root paste is applied topically on scabies and eruptions on the body. It is also used for preventing miscarriage. Traditionally, the root such plant was prescribed in combination with other drugs for the treatment of snake bite and scorpion sting.[3]

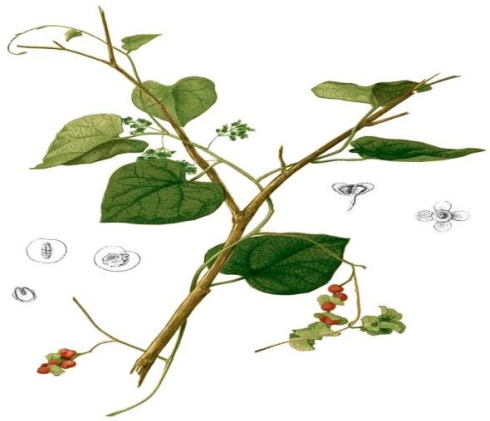

Fig.1: Aerial parts of *Cissampelos pareira linn* plant.

Fig.2: Rhizome of *Cissampelos pareira linn* plant.

Cissampelos pareira Linn is a potential herb. It is found that *Cissampelos pareira* has potential medicinal activity and can be used in the treatment of various diseases. By going throughout the literature review, various pharmacological activities of this plant has been familiarized and it is also found that plant contains a wide range of phytochemical constituents which need to be explored more and more so that the single constituent related activity can be performed.[4] It contains a number of alkaloids, especially bis-benzyisoquinoline alkaloids. The rhizome contains hayatine, hayatidine, hayatinine, d-4”-O-methyl bebeerine, l-bebeerine, iso-chondrodendrine, dicentrine, dehydro-dicentrine, insularine[5-7]; the rhizome and leaves contain cycleanine,[8] while cissampareine has been isolated from the whole plant[9] and the chalcone-flavone dimer cissampeloflavone from the aerial parts. The rhizomes have also been found to be a rich source of tropoloisoquinoline alkaloids. Pareirubrine A, pareirubrine B, grandirubrine, isoimerubrine and pareitropone have been isolated, all of which showed potent antileukaemic activity.

The traditional system of medicine being highly used in Nepal since historical time lead to the Ayurvedic period. Nepal has been mentioned as sacred heaven of potent medicinal and aromatic plants as the country is meeting place of western and Eastern Himalayan plants. About 1,624 plant species with medicinal value has known to Nepal. Based on field data collection, Manandar has reported more than 1,500 kinds of plant used in rural part of Nepal.[10] Significant numbers of traditional healers and their indigenous medical knowledge, recipes, technologies, herbal resources, minerals, animal parts etc are the important components and resources of traditional medicines. However, non-codified knowledge has been transferring from generation to generation as a tradition but has not recorded. Despite having immense potentialities to promote public health as well as to capture the national as

well as international markets, the country is still far behind to grab the opportunities utilizing available resources.[11]

WHO traditional Medicine Strategy 2002-2005, which reviewed the status of Traditional Medicine globally and in Member States and set out four key objectives i.e., Policy, Safety, efficacy, quality and rational use, access. WHO strategy helps to continue the challenge related to develop research and development, integration, in particular identifying and evaluating strategies and criteria for integrating traditional medicine international and primary health care.[12]

The present study was conducted to screen phytoconstituents and evaluate antibacterial activity of rhizomes of *Cissampelos pareira Linn* with an objective to analyze the potentiality of this plant as new potent antibacterial medicines which would boost the commercial value of the medicinal plants.

2. MATERIAL AND METHODS

2.1 Plant Materials

This study was conducted in the research laboratory of Crimson College of Technology, Butwal, Nepal during October 2017. The fresh part of *Cissampelos pareira* plant- rhizome was collected from Jhumsa, Palpa, Western region, Nepal that had been confirmed by our senior botanist.

2.2 Preparation of Crude Extract

The plant parts were collected, washed, shade dried at room temperature and later pulverized by mechanical grinder. The powder was passed through a 40-mesh sieve and stored in a closed vessel until use. The grinded rhizomes (Fig.3) (50 gram each) were double macerated with 250ml ethyl acetate and 250ml methanol at room temperature for 48 hours followed by addition 250 ml of each solvent in second maceration. The extracts were decanted, filtered through Whatman No. 1 filter paper and were collected in mouth closed beaker separately. It was then concentrated in reduced pressure at 30 ºC on rotary evaporator. The obtained crude extract was then collected in well labeled small beaker.

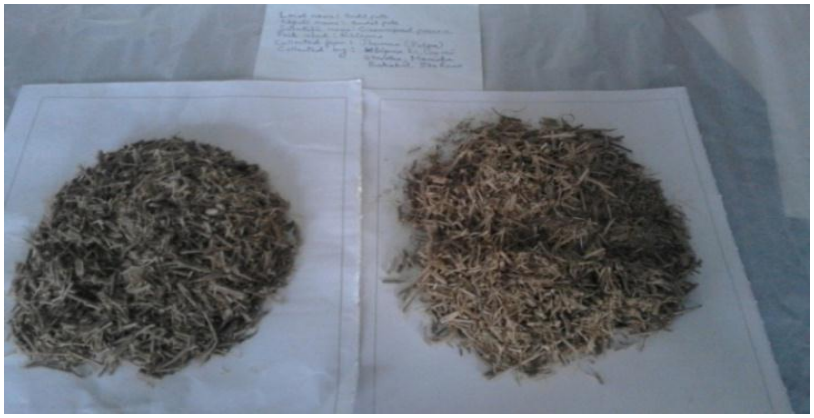

Fig. 3: Grinded form of *Cissampelos pareira* rhizomes.

2.3 Preliminary Phytochemical Screening & test procedure

The phytochemical screening of the methanolic and ethyl acetate extract of plant was conducted using standard procedures for the identification of various active constituents. The following qualitative tests were carried out.

Tests for Alkaloids

***Mayer's Test*:** A fraction of the extract was treated with Mayer's reagent and observed for the formation of a white precipitate or creamy colored precipitate, indicated presence of alkaloids.

Tests of Glycosides

***Baljet Test*:** To 1 ml each extract was added with 1 ml sodium picrate solution and the yellow to orange color revealed the presence of glycosides.

Test for Tannins and Phenolic compounds

Gelatin Test: A fraction of the extract was treated with 1% gelatin containing 10% NaCl and observed for the precipitation, indicated the presence of Tannins and Phenolic compounds.

Test for Saponins

About 1 ml of each extract was diluted separately with distilled water to 20 ml, and shaken in a graduated cylinder for 15 minutes. A1% 1 cm layer of foam indicated the presence of saponins.

Test for Fixed Oils

Spot Test: A small quantity of extract was pressed between two filter papers. Oil stains on the filter paper indicated the presence of fixed oils.

Test for Carbohydrates

Molisch's Test: To 2 ml of the extract, 1 ml of α- napthol solution was added, and concentrated sulphuric acid was added through the sides of test tube. Purple or reddish violet colour at the junction of the two liquids revealed the presence of carbohydrates.

Test for flavonois

***Sulphuric acid test*:** 2mg of dry sample was taken in the test tube. 2ml of concentrated sulphuric acid was added to it. Formation of a deep yellow or orange or red to bluish colored solution was indicative for the presence of flavonoids.

Test for cardiac glycoside

Glacial acetic acid test: To 5ml of the test solution in a test tube, 2ml of glacial acetic acid solution containing 1 drop of 5% ferric chloride solution was added to it. This solution was then carefully transferred to the surface of 1ml concentrated sulphuric acid. Formation of reddish brown ring at junction of two liquid was indicative for the presence of cardiac glycoside.

2.4 Antibacterial Assays

The antibacterial activity was assessed according to the standard procedure. Ciprofloxacin and Ampicillin antibiotics were used as positive control. This activity was evaluated on *Staphylococcus aureus* (gm positive) and *Escherichia coli* (gm negative) provided from National Patho-Lab, Butwal, Nepal.

2.4.1 Preparation of solution of extract

The extracts were dissolved in Dimethyl Sulphoxide (DMSO) to make a solution of concentration 2mg/ml of each ethyl acetate and methanol extract. Then mixed properly by vigorous shaking and covered with aluminium foil.

2.4.2. Sensitivity test

Bacterial suspension of *Staphylococcus aureus* and *Escherichia coli* was prepared by addition of nutrient agar. The Mular Hintong agar media was prepared in the conical flask. Then it was kept in an autoclave for 15 min at 121 ºC at 15 Ibs pressure. The autoclaved media was then poured into the petridisc and let them solidified under acceptable environment on refrigerator for 24 hr. Then the solution of test organism was inoculated on petridisc with media with the help of sterilized inoculating loop and streaked across the surface of medium.

A 10µL micropipette was adjusted so that a single press on the micropipette delivered a concentration of 200µg/ml of plant extract on filter paper discs. In the similar, 10mg/ml of DMSO was pipetted on filter paper discs. By this process, 16 discs were treated with 200µg/ml concentration of rhizome plant extract and let it to diffuse for 1hr. Following this, the small paper disc incorporated with standard antibiotic ciprofloxacin and ampicillin was also placed on such culture media. Total 16 petridisc with proper labeling was used, 8 petridisc for each single extract was used. The plates were then incubated at 37˚C for 24hr for bacteria. After the specified time, the zone of inhibition of extracts, antibiotics and DMSO were measured to the nearest millimeter using a ruler. The diameter of the disc was included in the measurement.

2.4.3 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MICs) were determined by using broth dilution method. The MIC is the lowest concentration at which the visible growth of a strain was completely inhibited (no visible turbidity in tube). The plant extracts were solubilized in DMSO solvent to prepare extract solution of concentration 500 mg/ml. The 2ml of extract (5mg/ml) was added to the test tube 1, containing additional 2ml of nutrient broth along with test organism (10µg/ml). After roughly mixing, 2.5ml of solution was transferred to second test tube and process was continued for succeeding transfers. The latest tube was received with no test solution and served as controlled. Such process was continued for each of solvents extract. Then, culture was kept in an incubator at 26˚C and result was observed after 24hr.

3. RESULTS

3.1 Extraction yield value

After double maceration process, the rhizome extracts of both solvent is obtained and their yield value is calculated. Among both extracts, methanolic extracts have higher yield value than ethyl acetate extracts (Table 1).

Table 1: Yields of different extracts of rhizomes of *Cissampelos pareira.*

Name of plant	Plant Part	Extract Yield value (%)	
		Ethyl acetate	Methanol
Cissampelos pareira	Rhizomes	3.2872%	8.5552%

3.2 Phytochemical screening

The Methanolic extracts of the rhizomes of *Cissampelos pareira linn.* plant have shown more positive tests for the presence of constituents during phytochemical screening than ethyl acetate extracts (Table 2).

Table 2: Phytochemical screening of Rhizomes of *Cissampelos pareira linn.*

S.N.	Type of Test	Reagent Use	Observation	Ethyl Acetate Extract	Methanolic Extract
1.	Alkaloid	Mayer test	Formation of white and cream precipitate	-	+
2.	Glycoside	Baljet reagent	Yellow to orange color	-	+
3.	Tannins and phenolic compound	Gelatin test	Formation of precipitate	-	-
4.	Saponin		Foam layer	-	-
5.	Fixed oil	Spot test	Oily stain	-	+
6.	Carbohydrate	Molisch test	Purple or reddish violet	-	+
7.	Flavonoids	Lead acetate test	A bulky white or yellowish precipitate	-	+
8.	Cardiac Glycoside	Glacial acetic acid test	Formation of reddish brown ring at the junction of two liquid	+	+

***Key**: (-)ve =Absence of constituent, (+)ve = Presence of constituents*

3.3 Antimicrobial activity test

Antimicrobial activity of different solvent extract of *Cissampelos pareira linn* rhizomes at concentration 200mg/ml and zone of inhibition in mm were measured. The Ethyl Acetate extract showed the highest inhibitory action against *S. aureus* in comparison to methanolic extract whereas the methanolic extract in turn showed higher inhibitory action against *E. coli* than Ethyl acetate extract (Fig. 4).

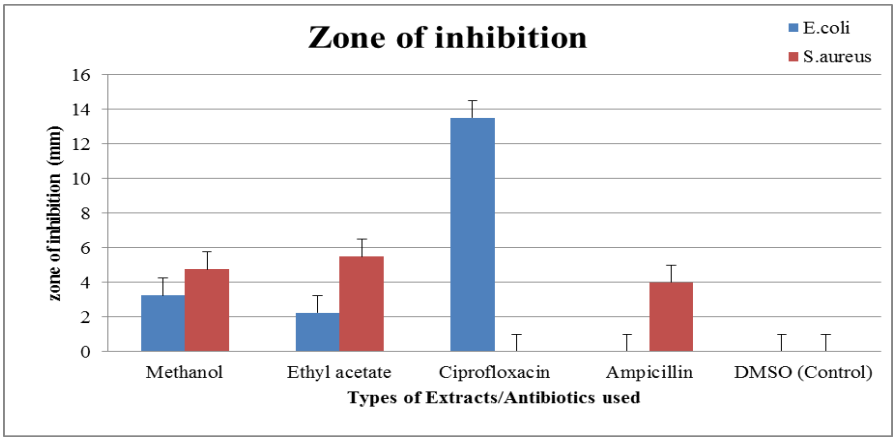

Fig. 4: Zone of inhibition of methanolic and ethyl acetate extract and antibiotic against *E. coli* and *S. aureus.*

Following the broth dilution method, the minimum inhibitory concentration (mg/ml) was determined after successive dilution of plant extract. It was found that the MIC value of methanolic extract & ethyl acetate extract of plant rhizome against E. coli & S. aureus was lower that suggested greater susceptibility of such organisms towards plant extract respectively (Table 3).

Table 3: Minimum Inhibitory Concentration of rhizome extract.

S.N.	Type of extract (200 mg/ml)	Minimum inhibitory concentration (mg/ml)	
		S. aureus	***E. coli***
1	Methanol	1.25	0.56
2	Ethyl Acetate	0.62	1.25

4. DISCUSSION

Cissampelos pareira Linn is a potential herb belongs to the family Menispermaceae. Numbers of species are available throughout the world. It is concluded that *Cissampelos pareira* have potential medicinal activity and can be used in the treatment of various diseases. By going through literature review, various pharmacological activities of this plant have been familiarized and it is also found that plant contains a wide range of phytoconstituents. The use of plants has been in existent since prehistory.[2]

Cissampelos pareira is a very useful herb for women's affections. It has been used since the ancient times as a cure for uterine bleeding, due to abortion, leucorrhoea and vaginitis. The decoction of its bark is given orally or in form of pessaries/suppository (basti) as well. The latex admixed with sugar removes sexual debility in males. The powdered bark works well as an anorexient.[13]

In another research, MP et al. carried out a study on the leaf of *Cissampelos pareira* plant that showed the phytochemical analysis on leaf extract by different solvent system. The leaves were extracted in five different solvents such as Aqueous, Methanol, Ethyl acetate, Acetone and hexane. The phytochemical test for constituent like Acids, betacyanin, quinones, coumarins, carbohydrates, alkaloids, amino acids, fixed oils and fats, flavonoids, steroids, tannins, resins, terpenoids, phenols, cardiac glycosides, volatile oils and starch present in different extracts of leaf are performed in different solvent system i.e. aqueous, hexane, ethyl acetate, alcohol, methanol. Among such tests, solvent aqueous indicates presence of all constituent but absence of constitution like carbohydrate, alkaloid, tannin, phenol, saponins, and cardiac glycoside. The solvent hexane indicates the absence of alkaloid, fixed oil, tannin,

phenol, saponin, cardiac glycoside. Solvent ethyl acetate shows absence of carbohydrate, alkaloid, fixed oil, flavonoid, saponins. Alcohol shows absence of carbohydrate, fixed oil, flavonoid, phenol, saponin. The methanolic extract indicates absence of carbohydrate, flavonoid, phenol, saponin, fixed oil. Except these constituent as mentioned above, others are present.[14]

Similarly, antifertility activity was reported by Ganguly et al in *C. pareira* leaf extract who found the consequences that when administered orally, altered the estrous cycle pattern in female mice, prolonged the length of estrous cycle with significant increase in the duration of diestrus stage and reduced significantly the number of litters in albino mice. The analysis of the principal hormones involved in estrous cycle regulation showed that the plant extract altered gonadotropin release (LH, FSH and Prolactin) and estradiol secretion. The oral LD50 of the extract was found to be 7.3 g/kg in mice.[15]

The similar type of study was carried out by Morita et al. that focused on isolation of active constituents from roots found in different geographical regions. During his investigation, he reported that tetrandrine was present in the roots of *Cissampelos pareira* growing in Thailand. Similarly, dicentrine, dihydrodicentrine, cycleanine, insularine and isochondrodendrine had been reported from roots of the plant growing in Ghana. Isolation of pareirubrine A and B, novel tropoloisoquinoline alkaloids with antileukaemic activity had been also reported.[16]

In our study, the *Cissampelos Pereira*'s rhizomes extracted in two different solvents were evaluated for phytoconstituents present in them. Based on photochemical screening, the plant extracts contain alkaloids, glycoside, fixed oil, carbohydrate, glacial acetic acid, sulphuric acid, flavonoid. The present solvent extracts of *Cissampelos pareira* contain medicinally improved bioactive compounds. This justified the use of plant species as traditional medicines for various ailments. It was found that the methanolic extract had the highest yield value (8.5552%) as compare to ethyl acetate extract (3.2872%).

Thus, during phytochemical screening, the methanolic extract of the plant rhizome showed many positive results for presence of phytoconstituents whereas ethyl acetate rhizome extract showed less positive results. The Methanolic extract indicated presence of constituents like alkaloid, glycoside, fixed oil, carbohydrate, flavonoid, cardiac glycoside but it indicated absence of saponin, tannin and phenolic compound. The Ethyl acetate extract showed

presence of cardiac glycoside constituent and absence of alkaloid, glycosides, tannin and phenolic compound, saponin, fixed oil, carbohydrate, flavonoid.

The present study of *Cissampelos pareira* plant rhizome determined the antibacterial activity. The antimicrobial activities were performed against the organism *Escherichia coli* and *Staphylococcus aureus* at concentration 100µg/ml which did not shown activity. But when concentration increased to 200µg/ml, the antimicrobial activity was observed. The methanolic extract exhibited antibacterial sensitivity against both organism used and so did ethyl acetate extract.

The Methanolic extract indicated the maximum zone of inhibition at 1mg/disc at concentration of 200mg/ml. The maximum zone of inhibition for *S. aureus* was 5.5±1.290mm with ethyl acetate extract and for *E. coli* was 2.25±0.5mm. In case of Methanolic extract, the maximum zone of inhibition for *S. aureus* was 4.75±1.8929mm and for *E. coli* was 3.25±0.9574mm. Thus, the present study showed that the *S. aureus* i.e. gram positive bacteria was more susceptible than the *E. coli*.

5. CONCLUSION

The phytochemical analysis conducted on plant part (rhizome) determined the presence of bioactive compounds like alkaloid, glycosides, fixed oil, carbohydrate, flavonoids and glacial acetic acid. In phytoscreening analysis, the double maceration method was used for the extraction process with help of two solvent system i.e. ethyl acetate and methanol followed by different standard tests to determine the phytoconstituents present in plant rhizome. In our study, the extracts of *Cissampelos pareira* demonstrated antibacterial activity against both gram-positive and gram-negative bacteria. The broad-spectrum antibacterial activity of the plant extract was possibly due to the identified chemical constituents. The antimicrobial test result showed that the extracts of such plant rhizome exhibited antimicrobial activity as well as the susceptibility of *E. coli* and *S. aureu*s organism towards plant rhizome extract.

The study of various bioactive substances from our research work and also by other research activities conducted on this plant recommends that it has possible use in formulation of antimicrobial agents for the treatment of many diseases. Isolation, identification and purification of these phytoconstituents and determination of their respective antimicrobial potencies and toxicological evaluation with the view to formulating novel chemotherapeutic agents should be the future direction for investigation.

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7. REFERENCES

1. Saxena M, Saxena J. Phytochemical Screening of *Acorus calamus* and *Lantana camara.* International Research Journal of Pharmacy, 2012; 3(5): 324-26.
2. Arora M, Bansal N, Devi A, Sharma T, Siddiqui AA. An Inside Review of *Cissampelos pareira Lin.*: A Potential Medicinal Plant of India. International Research Journal of Pharmacy, 2012; 3: 38-41.
3. Jogi BV, Mistry HA, Patel VG, Shukla SH. Pharmacognostical Standardization of Preliminary Phytochemical Screening of *Cissampelos pareira* Lin. Var. Hirsuts Root. Journal of Pharmacognosy and Phytochemistry, 2014; 2: 27-31.
4. Jain SK, Ahirwar SK, Kumar A. *Review of Cissampelos pareira linn.* International Journal of Applied Research, 2015; 1(6): 08-09.
5. Bhattacharji S, Sharma S, Dhar VN, ML. Chemical examination of the roots of Cissampelos Pareira. J Sci Ind Res-B, 1956; 15: 363.
6. Bhatnagar AK, Bhattacharji S, Roy AC, Popli SP, Dhar ML. Chemical examination of the roots of *Cissampelos pareira*. IV. Structure and stereochemistry of hayatin. J Org. Chem, 1967; 32(3): 819-20.
7. Bhatnagar AK, Popli SP. Chemical Examination of the Roots of *Cissampelos pareira* Linn.: Part III - Structure & Stereochemistry of Hayatinin. Indian Journal of Chemistry, 1967; 5: 102.
8. Chowdhury AR. Chemical investigations on *Cissampelos pareira*. Sci Cult, 1972; 38: 358.
9. Kupchan SM, Patel AC, Fujita E. Cissamparein, a new cytotoxic alkaloid from *Cissampelos pareira*, cytotoxicity of bisbenzyisoquinoline alkaloids. J Pharm Sci, 1965; 54: 580.

10. Manandar NP. Plants and people of Nepal. Oregon, U.S.A.; Timber Press Portland: 2002, 154-155.
11. Koirala RR, Khaniya BN. Traditional medicine and medicinal and aromatic plant related organization in NP.1st ed., Ram shah Path, Kathmandu, Nepal; NP Health Research Council, 2009; 1-13.
12. WHO Traditional Medicine strategy 2002-2005. Geneva, 2002.
13. Poongothai A, Sreena KP, Sreejith K, Uthiralingam M, Annapoorani S. Preliminary phytochemicals screening of *Ficus racemosa* linn. Bark. Int. J. Pharma Bio Scs, 2011; 2(2): 431-34.
14. Anupa MP, Vasanthakumari B, Thavasimuthu C. Qualitative phytochemical screening of leaf extract of *Cissampelos pareira linn* in different solvent system. Int. J. Bioassays, 2014; 3(10): 3399-403.
15. Ganguly M, Borthakur M, Devi N, Mahanta R. Antifertility activity of the methanolic leaf extract of *Cissampelos pareira* in female albino rats. J. Ethnopharmacol, 2007; 111: 688-91.
16. Morita H, Mastumoto K, Takeya K, Itokawa H, Iitaka Y. A Novel Antileukaemic Tropoloisoquinoline Alkaloid, Pareirubrine, from *Cissampelos pareira.* Chem. Lett, 1993; 2: 339- 42.