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A Study on Phytochemical and Biological Screening of the Rhizome of *Asparagus racemosus*

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The objective of this study was to evaluate the biological screening, specially focused on antibacterial and cytotoxicity (Brine shrimp bioassay). Rhizomes of *Asparagus racemosus* were dried under shade and then powdered, and extracted with methanol and chloroform. Preliminary phytochemical studies were carried out in lab followed by TLC behavior with simple techniques prevailing the presence of flavonoid, tannins, quinines, saponin glycosides, terpenoid and steroid. TLC behavior was observed in UV light then visualized by spraying vanillin reagent and R_f value was found 0.388 and 0.336 in methanol and chloroform extract respectively. The antimicrobial test was performed by making 6 and 12% of extract in DMSO against the standard Amoxicillin and Chloramphenicol. Eight different bacteria (*S. aureus, E. coli, K. pneumonia, P. aeruginosa, S. dysentriae, B. subtilis, Proteus, S. paratyphi*) were taken among which zone of inhibition (ZOI) is more prominent in *B. subtilis* as compared to Amoxicillin. *E. coli, Proteus,* and *S. paratyphi* shows ZOI only in chloroform extract while *Pseudomonas spp* shows only in methanol extract. Brine Shrimp (*Artemia salina*) bioassay was carried to observe the lethality effect in high concentration i.e. in 1000 ppm, the extract was found to be cytotoxic. Our results allow us to conclude that the crude extracts of methanol and chloroform of the plant revealed antibacterial potential due to the presence of many phytochemical constituents, although the inhibitory activity was strain specific and concentration dependent. At higher concentration, both extract shows cytotoxic effect; hence the plant shows its great therapeutic effect.

Keywords: *Asparagus racemosus*, Antimicrobial, Brine shrimp (*Artemia salina)* Phytochemical and TLC.

INTRODUCTION

The revival of interest in natural drugs started in last decade mainly because of the wide spread belief that green medicine is healthier than synthetic products. Nowadays, there is manifold increase in medicinal plant based industries. Due to the increase in the interest of medicinal plants throughout the world which are growing at a rate of 7- 15% annually, despite the major advances in the modern medicine, the development of new drugs from natural products is still considered important. Medicinal plants as a possible therapeutic measure has become a subject of active scientific investigations.

Asparagus racemosus (called *Shatavari* in Ayurveda where it is established as Rasayana) is an herb, [1] and have also been reported to be used in Siddha and Unani medicine.[2] *Asparagus racemosus* (family Liliaceae) is locally known as Kurilo (Nepali).[3] It is herbaceous, perennial plant. The stem is woody pale grey or brown in color and armed with strong spines. Leaves are needle like sub erect, soft spines, the root stuck are tuberous, bearing numerous fusiform, succulent tuberous roots are 30-100 cm long and 1-2 cm thick and flowers are hermaphrodite and flowering season is October- November. Distributed 100-2100m East to West in Nepal.[3]

In Ayurveda, this amazing herb is known as the "Queen of herbs", because it promotes love and devotion. Shatavari is the main Ayurvedic rejuvenative tonic for the female, as is Withania for the male. It is effective in treating madhur rasam, madhur vipakam, seet-veery am, som rogam, chronic fever and internal heat.[4, 5] This herb is highly effective in problems related with female reproductive system. Charak Samhita written by Charak and Ashtang Hridyam written by Vagbhata, the two main texts on Ayurvedic medicines, list *Asparagus racemosus* (*A. racemosus*) as part of the formulas to treat women's health disorder.[6-9] A. racemosus is a well known Ayurvedic rasayana which prevent ageing, increase longevity, impart immunity, improve mental function, vigor and add vitality to the body and it is also used in nervous disorders, dyspepsia, tumors, inflammation, neuropathy, hepatopathy.

Conceivable biological action of Asparagus racemosus may be due to presence terpenoid, steroids, glycoside, saponin glycosides, flavonoid, flavonic glycosides, flavones aglycon, tannin, quinines, polyoses, and reducing compounds.[10]

The traditional use of medicinal plants has a long history. Ancient people as well as our ancestors were mainly dependent on plants for

their recovery against diseases. Therefore the present study a traditional plant medicine; *Asparagus racemosus* has been investigated for the Biological screenings because of their effectiveness, less side effects and relatively low cost.

MATERIALS AND METHOD

Plant Material

The herbs of *Asparagus racemosus* was collected from the Terai region of Nepal – Bardibas, Mohattari. The sample was identified by Scientist incharge, at National Herbarium and Plant Laboratory (DPR), Godawari, Kathmandu, Nepal.

Extraction of the plant materials and sample preparation

The fresh collected rhizomes were first boiled in water for 45 min thereafter washed, peeled and cut into small pieces, dried in hot air oven at 50^{o}c. dried plant materials were grinded up to coarse powder using grinder and then was weighed. Plant material was then ready for extraction process.

Cold maceration (with chloroform solvent) the plant materials were fully suspended in chloroform and were shaken well so that all the particles were suspended. The samples were kept suspended for 72 hours, it was considered as the first wash with chloroform. The process was repeated for second and third wash of sample. Second wash was completed after suspending the sample for 48 hours and third wash was completed after suspending extract for 24 hours. All the extracts were mixed, filtered and dried in water bath not exceeding 50^{o}c.

Soxhlet method (with Methanol solvent) the powder was used for the preparation of methanolic extract. Dried and powdered sample was extracted with boiling 70% MeOH in a reflux condition. After filtration, the extract obtained was concentrated in a rotary shaker and evaporated to dryness to get constant weight. [11, 12]

The qualitative phytochemicals screening were carried out on the chloroform and methanolic extract of *Asparagus racemosus* and further performed antimicrobial and cytotoxic analysis.

Experimental design

Brine Shrimp Assay[13]

Preparation of the test sample: Stock solution was prepared by dissolving 100 mg of extract in 25 ml of suitable solvent depending upon its solubility i.e. 4000 ppm. The appropriate solvent was little amount of DMSO for initial solubilisation and then addition of water. The stock solution was further diluted to make of 1000, 100, 10 ppm.

Preparation of Artificial Sea Water: Artificial Sea Water was prepared by dissolving NaCl (23.5 g/l), KCl (0.68 g/l), $NaHCO_3$ (0.196 g/l), Na_2SO_4 (4.001 g/l), Boric acid (0.027 g/l), $CaCl_2$ (1.780 g/l), $MgCl_2$ (10.680 g/l) and sodium EDTA (0.003 g/l).

Hatching of Brine Shrimp: 50 mg of Brine Shrimp eggs were sprinkled in a beaker with 900 ml of sea water. The transferred sample was then allowed to incubate at 32-35^{o}c and light was also provided for 24 hrs.

Bioassay: Cleaned test tubes were divided into 5 groups, first 2 groups' consisting of 6 test tubes. After 24 hrs of incubation, the nauplii were recovered with a pipette and 10 nauplii were transferred in each test tube. The groups were administered with different dilutions of sample and the test tubes were then incubated at 32-35^{o}c overnight. The incubated test tubes were observed for the number of survived nauplii for 24 and 48 hours.

Antimicrobial Assay:

Standard Antibiotics (Chloramphenicol 30mcg: Hi media lab, Mumbai) and Amoxicillin (30 mcg: Hi media lab, Mumbai).

Micro-organisms used; *Staphylococcus aureus, Escherichia coli, Kliebsiella pneumonia, Pseudomonas aeruginosa, Shigella dysentriae, Bacillus subtilis, Proteus, Salmonella paratyphi.*

Antimicrobial Screening

Preparation of sample: The extraction of plant was dissolved in DMSO, which was made into different concentrations viz. 6%, 12% for methanol and 6%, 12% for chloroform as well.

Preparation of the Inoculums: For the preparation of the bacterial suspension, slants of nutrient agar were prepared by pouring 10 ml of the medium in test tubes and were kept in slanting position. The Medias were let to solidify for 15 min. Then with loop, a colony of each bacterium were streaked in the different slants and then incubated for 24 hrs at

37°c in an incubator. In the prepared slants 10 ml of freshly prepared sterile saline solution was added and the colonies formed on the medium were scraped with an inoculating loop. Turbid solution of each bacterium was obtained and kept for further use. All these activities were carried out in horizontal laminar flow. [14]

Preparation of the culture media: Well in agar method was used for the preparation of culture media. Muller Hinton Agar (MHA) was used to prepare bacterial culture media to study the antibacterial sensitivity test. The MHA culture media was prepared at the range of 38 gm media in 1000 ml of DW. The solution obtained after mixing the media in DW was heated to boiling in order to ensure the proper mixing. The media in the conical flask was then covered with cotton. The media was sterilized in autoclave; 20 ml of agar media were poured in sterilizes petriplates having the diameter of 9 cm. After the solidification of the media, each bacterium was spread in each petriplates with the help of cotton swab, after 30 min 4 bores were made in each plate with the help of a borer having the diameter of 6mm for the plant extracts while a single bore was made for the standard antibiotics. For an extract 16 plates were prepared in order to investigate the sensitivity in 4 different concentrations against different organisms. Then 100 µl of each extract were poured in the respective bores and plates. Then the plates were left in the laminar flow for 1 hour to diffuse the extract and antibiotic solutions in the media. The plates were then incubated for 24 hr at 37°c for bacteria. After the respective time the ZOI of the extract and the antibiotics was measured.[14]

TLC behaviour of extract

Preparation of solvent system: Ethyl acetate: Methanol: water (75 ml: 15ml: 10 ml) respectively. The detection of spots in the TLC plates was carried out by visualization under UV light before spraying vanillin sulphuric acid reagent. [15]

Statistical analysis

The results are expressed as mean ± standard error of mean and one-way analysis of variance (ANOVA).

RESULTS

Extraction yields

The extract yields of the crude drugs are Table 01

S.N.	Samples	Yield % Methanol Extract	Yield % Chloroform extract
1.	*Asparagus racemosus*	46.48%	0.9 %

Preliminary phytochemical study

Table 02 Preliminary Qualitative analysis of *Asparagus racemosus* was analyzed. It was observed that the mixture contains Glycosides, Flavonoid, Flavonic acid, Quinones, Polyoses and Reducing compounds (Sugar).

Samples (*Asparagus racemosus*)			
S.N.	**Constituents**	**Chloroform extract**	**Methanol extract**
1.	Alkaloids(Mayer's test)	-	-
2.	Steroid and Triterpenoids A) Leibermann's Test B) Salkowski Test	A) - B) -	A) - B) -
3.	Glycosides (Molisch test)	-	+
4.	Glucosides	-	-
5.	Anthocynosides	-	-
6.	Cardiac glycosides (Keller-Killani test)	Not Feasible	-
7.	Saponins glycosides	-	+
8.	Anthraquinone glycosides (Borntrager's test)	-	-
9.	Flavonoids, Flavonic glycosides, Flavones aglycone	-	+
10.	Tannins	+	-
11.	Quinones	+	+
12.	Coumarins	Not Feasible	-
13.	Carotene	-	-
14.	Polyoses (Carbohydrates)	-	+
15.	Anthracenoids or Emodins	-	-
16.	Reducing compounds (Sugars)	-	+

TLC behaviour of extract

TLC behaviour was observed in UV (254nm) before spraying the vanillin. After the spraying, clear spots were observed and their R_f value was calculated – Table 03

S.N.	Samples	Spots observed in Methanol extract (cm)		Sports observed in Chloroform extract (cm)		Mean value of ME	Mean value of CE
		N = 1	N = 2	N = 1	N = 2		
1.	*Asparagus racemosus*	4.7	5	4.2	4.2	4.85	4.2

- R_f value for methanol extract; 0.388 and chloroform extract 0.336.

Fig.1: TLC of *Asparagus racemosus* after vanillin spray

Fig.2: Observation of TLC in UV before vanillin spray

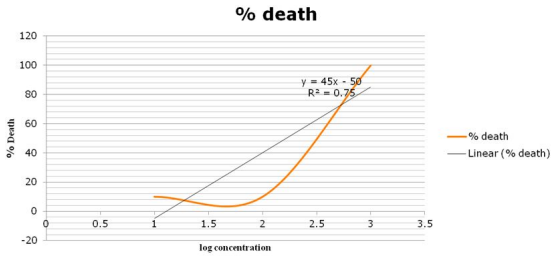
Brine Shrimp Bioassay

Brine Shrimp Bioassay was carried out to observe the cytotoxicity effect of plant material and it was found that in high concentration the plant material posses the characteristic of cytotoxic.

Table 04: Brine Shrimp assay for chloroform and methanol extracts

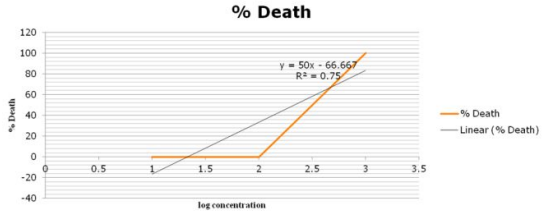
Extract	Extract conc. In ppm (A)	Log (A)	Initial Nauplii	Nauplii survival n=1	Nauplii survival n=2	Mean survival	Death	% Death	% survival
Methanol	10	1	10	10	8	9	1	10%	90%
	100	2	10	9	9	9	1	10%	90%
	1000	3	10	0	0	0	10	100%	0%
Chloroform	10	1	10	10	10	10	0	0%	100%
	100	2	10	10	10	10	0	0%	100%
	1000	3	10	0	0	0	10	100%	0%
Standard		1	10	0			10	100%	0%
A) Distilled Water (D/W)									
B) Seawater (S/W)		1	10	8			2	20%	80%
C) DMSO (with sea water)		1	10	10			0	0%	100%

Graph 01: Log of concentration VS % death (for methanolic extract)



The equation of line: y = 45x – 50
For LC_{50} determination, we take y = 50, so putting y = 50 in equation
We get,
x = 2.222
I.e. antilog (2.222) = 166.72
I.e. the LC_{50} for methanolic extract was found to be 166.72 ppm

Graph 02: Log concentrations VS % death for chloroform extract



The equation of line: y = 50x – 66.667
For LC_{50} determination, we take y = 50, so putting y = 50 in equation
We get,
x = 2.334
Antilog (2.334) = 215.77
I.e. For chloroform extract LC_{50} was found to be 215.77 ppm.

Antimicrobial Assay

Zone of Inhibition was used for the screening of antibacterial properties. *E. coli, Proteus, B. subtilis, S. paratyphi and P. aeruginosa* was found to be sensitive to the plant extract.

Table 05: ZOI of standard antibiotics against different bacterial strains

Zone of inhibitions of Chloroform and methanol extracts on different pathogenic microbes

S.N.	Bacterial strains	Standard antibiotics used	ZOI (cm)
1	*E. coli*	Amoxicillin	1
		Chloramphenicol	2.9
2	*Proteus*	Amoxicillin	1.5
		Chloramphenicol	2.3
3	*K. pneumonia*	Amoxicillin	0
		Chloramphenicol	1.7
4	*S. paratyphi*	Amoxicillin	1
		Chloramphenicol	2.7
5	*S. aureus*	Amoxicillin	2.9
		Chloramphenicol	3.3
6	*B. subtilis*	Amoxicillin	0.6
		Chloramphenicol	2.9
7	*S. dysenteriae*	Amoxicillin	1.7
		Chloramphenicol	2.9
8	*P. aeruginosa*	Amoxicillin	0
		Chloramphenicol	2.8

Table 06: ZOI of different extracts against different bacterial strains

S.N.	Bacterial strains.	ZOI of Methanol Extract (cm)		ZOI of Chloroform Extract (cm)	
		6%	12%	6%	12%
1.	*Escherichia coli*	-	-	0.8	0.9
2.	*Proteus*	-	-	-	0.8
3.	*Klebsiella pneumonia*	-	-	-	-
4.	*Salmonella paratyphi*	-	-	-	0.7
5.	*Staphylococcus aureus*	-	-	-	-
6.	*Bacillus subtilis*	0.8	1.1	0.9	1.1
7.	*Shigella dysenteriae*	-	-	-	-
8.	*Pseudomonas aerogenosa*	-	0.8	-	-

Zone of Inhibition of different extract against bacterial strains

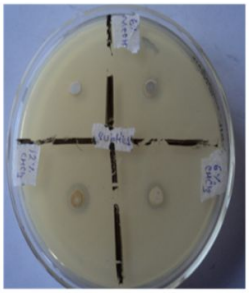


Fig 3: ZOI of chloroform extract and against *B. subtilis*

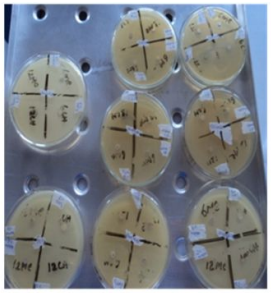


Fig 4: ZOI shown by *extracts* against taken bacteria

DISCUSSION

In the present era, plant and herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization. Although a significant number of studies have been used to obtain purified phytochemicals, very few screening programs have been initiated on the crude plant materials.[16]

The present study determined the antibacterial activity of *A. racemosus* plants from eastern part of Nepal. Two different solvent (Methanol and Chloroform) were used for extraction. It was seen that the extraction yield in methanol was higher than that of chloroform. The highest extraction yield for methanol was 46.48% while for chloroform was 1%. Factors like the age of the plant, geographical distribution and polarity of the solvent used may affect the yield of the plant extracts.[17]

In the present investigation, the antibacterial activities of the plant extracts were tested against eight microorganisms *S. aureus, E. coli, K. pneumonia, P. aeruginosa, Shigella, Bacillus, Proteus and S. paratyphi* at two different concentrations (6% and 12%). Both methanol and chloroform extract showed found to be effective against tested strains. Chloroform and methanol extract showed more pronounced antibacterial activity against *B. subtilis* as well methanol and chloroform both extract showed nearly similar antibacterial activity against *B. subtilis.* Gram negative bacteria were found to be more susceptible than gram positive bacteria from the experiment carried out.

Appearance of different spots on TLC plates by subjecting to UV chamber, after spraying vanillin with conc. H_2SO_4 indicated the presence of secondary metabolites. These compounds have potential application against human pathogens and several authors have linked the presence of these bioactive compounds to the antibacterial properties. Factors like the age of the plant, geographical distribution and polarity of the solvent used may affect the yield of the plant extracts.[18]

The Brine Shrimp test (BST) represents a rapid, inexpensive and simple bioassay for testing plant extract lethality which in most cases correlates reasonably well with cytotoxic and antitumor properties. 65 for chloroform extract cytotoxicity found was to be in 166.72

ppm while in methanol it was 215.77 ppm. In other words, mortality increased gradually with the increase in concentration of the plant extract.

CONCLUSION

The finding of this study indicates that crude extracts of *A. racemosus* revealed the presence of bioactive components as tannins, phenolics, anthraquinones, saponins and flavonoids. *Asparagus racemosus* showed antimicrobial activity against almost all test bacteria, *E. coli*, *Proteus, Salmonella paratyphi, Bacillus subtilis and Pseudomonas aeruginosa*. Chloroform extract of *A. racemosus* had showed potential activities against most of bacteria taken. Gram negative bacteria were found to be more susceptible than Gram positive bacteria from the experiment carried out. For chloroform extract cytotoxicity found was to be in 166.72 ppm while in methanol it was 215.77 ppm. In other words, mortality increased gradually with the increase in concentration of plant extract.

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Conflict of Interest: Certify that we have no conflict of interest in the subject matter.

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