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IJPBS |Volume 3| Issue 2 |APR-JUN |2013|99-107

Research Article
Pharmaceutical Sciences

IN VITRO EVALUATION OF ANTI BACTERIAL ACTIVITY OF EXTRACTS FROM *CASSYTHA FILIFORMIS LINN* AGAINST UROGENITAL CLINICAL GRAM-NEGATIVE BACTERIA

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ABSTRACT

The antibacterial properties of extracts from aerial parts of Cassytha filiformis Linn were evaluated. The plant part was shade-dried, pulverized and extracted with hot-water, methanol and n-hexane. Hot-water extraction was by 24 h maceration while extraction with methanol and n-hexane was done in a Soxhlet apparatus. The extracts were, then, concentrated by evaporation to dryness in an oven at temperature of 40-45^{0}C.. Preliminary antimicrobial Screening of these extracts was carried out using agar-well diffusion techniques. Gentamicin (Gentalek ^{R}) was used as reference standard antibiotic. The minimum inhibitory concentrations (MIC) of the extracts and the standard reference antibiotics were determined using agar dilution technique. The methanol extract of the cassytha filiformis has no activity against Proteus mirabilis and Klebsiella sp.The respective mean MIC values of the methanol extract from Cassytha filiformis Linn against Escherichia coli and Pseudomonas aeruginosa were 14.42 ± 0.58 mg/ml and 13.33 ± 1.1 mg/ml while for hot water extract were 21.35 ± 2.38 mg/ml, and 10.83 ± 1.32 respectively. The extracts (except the n-Hexane extract) from the aerial part of casytha filiformis are active against gram negative bacteria .The antimicrobial activities of these extracts could be attributed to the presence of those phytochemicals with good antimicrobial potentials.

KEY WORDS

Antibacterial activity, Cassytha filiformis, Urogenital Clinical Gram negative bacteria, MIC, Agar dilution technique.

INTRODUCTION

Cassytha filiformis has pantropical distribution throughout coastal areas (Scot, 2008) .They are perennial parasitic and seed-bearing plants. Seeds many be spread by Animal, water, strong winds, farm machinery or with crop seed. The plant may spread locally by vegetative growth between hosts and over soil. In modern medical research, *C. filiforms* has a number of biologically active chemical coupounds with potential human health application. For instance, ocoteine isolated from *C. filiformis*, as an alpha–adrenoceptor antagonist in rat thoracic aorta, have antiplelet aggregation activity (Chang et al 1997). *Cassytha filiformis* is used as Vasorelaxant (Wu; et al 1998), and adrenoceptor antagonist (Hoet et al, 2004) antitrypanosomal agent (Chang et al 1997) and diuretics in traditional medical practice (Kirtikar and Basu 1991).

Pytochemical screening reveals that the aerial parts of *Cassytha filiformis* consists of alkaloids, flavonoids, triterpenoid, and steroids (Sharma et al, 2009). Other reports show that some of the isolated compounds from this plant are lignan, cassyformin, filiformin, apomorphine alkaloids, actinodophine and octeine (Chang Fang-Rong et al, 1998)

The urogenital tract consists of those organs and glands that are involved in production, storage and discharge of Urine and those organs involved in reproduction. Urogenital infection occurs when micro-organisms multiply and cause infection of one or more of these organs. These micro-organisms may be bacteria or fungi or both.

Urogenital infection is caused by *Escherichia coli (the* commonest pathogen isolated*), proteus spp, Staph.aureus* (coagulase positive), coagulase negative *Staph spp, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa and Candida albicans* in immuno compromised or diabetic patients. Other occasional causative micro-organisms include *Citrobacter, Salmonellae*, *Streptococus pyogenes, S. agalactiae* and *Ureaplasma urealyticum* (Arora,1999)

E.coli is the commonest organism causing UTI. Most frequently encountered O serotypes of *E.coli* in UTI include 01, 02, 04, 06, 07, 018 and 075. These are also known as nephrotoxigenic strains. Special nephropathogenic potentials of these strains appear to be due to the polysaccharides of the O and K antigens which protect the organism from the bactericidal effect of complement and phagocytes in the absence of specific antibodies. E.coli that causes UTI often originates in the gut of the patient. The bacteria may gain access to the urinary tract by the ascending or the haematogenous route. The bacteria from the faecal flora spread to the perineum and from there they ascend into the bladder.

Proteus mirabilis is the most important species (70 – 90%) recovered from human, particularly as a causative agent of UTI and wound infections(Arora, 1999). It commonly involves young boys and elderly people of both sexes often with diabetes or structural abnormalities of the urinary tract. In the hospital patients, it may lead to UTI following various forms of urological instrumentation. UTI infection caused by proteus tends to be more serious than that caused by *E. coli* and other Coliforms, because these organisms are usually confined to the bladder whereas proteus sp has a predilection for the upper urinary tract. It produces urease which liberates ammonia from urea. Ammonia inactivates complement, damages renal epithelium and makes the urine alkaline. The alkaline conditions lead to the precipitation of phosphates and the formation of calculi in the urinary tract.

Klebsiella have been incriminated in nosocomial infections. Common sites include the urinary tract, lower respiratory tract, biliary tract and surgical wound sites. The spectrum of clinical syndromes includes pneumonia, bacteria urinary tract infection (UTI), diarrhea, upper respiratory tract infection, wound infection osteomyelitis and meningitis

P. aeruginosa is a major opportunistic bacteria pathogen of nosocomial infections. The mult resistant strains play an important role in the colonization or infection of chronically hospitalized patients. It causes urinary tract infection when it is mechanically placed into the urinary tract during catheterization (Arora, 1999). This study was conducted to evaluate the antibacterial potentials of extracts from cassytha filiformis Linn against urogenital clinical gram-negative bacteria.

MATERIALS AND METHODS

PLANT MATERIAL

The aerial parts of *Cassytha filiformis Linn* were collected from Nsukka in Enugu State between June and August 2009. The identity of the two plants were authenticated by Mr. A .O .Ozioko of the Bioresource Development and Conservation programme (BDCP) Nsukka, Enugu State. extraction processes.

Test micro organisms

A total forty four (44) urogenital Gram negative bacteria were used for the study- 12 isolates of *Klebsiells spp*, 13 isolates of *Escherichia coli*, 10 isolates of *Proteus mirabilis*, and 9 isolates of *Pseudomonas aeruginosa*. They were isolated from uro-genital specimen [urine, high vaginal swab (HVS), endocervical swab (ECS), and urethral Swab (US) from patients who were visiting the Laboratory Department of Bishop Shanahan Hospital at Nsukka, Enugu State. These patients were manifesting signs and symptoms of uro-genital infections (Arora 1999)

Chemicals and Media

The culture media, chemicals,solvents and reagents used were of analytical grade and were products of ANTEC diagnostic product, England ,Fluka Biochemika, Germny , BIOTECH, United Kingdom. Riedel-deHaen and SIGMA ALDRICH, Germany

Antibiotics used was gentamicin (Gentalek^{R}) [Taylek Drug Limited, India].

Extraction of plant material

The chopped aerial parts of the *Cassytha filiformis* Linn were shade-dried. The dried plant materials were pulverized into powder and the powder weighed with Triple beam balance (MB, 2610, China). A 400 g of *Cassytha filiforms Linn* was macerated with 4.0 L of hot water and allowed to stand at room temperature for 24 h. The extracts were filtered using porcelain sieve. These hot-water extracts were concentrated by evaporation to dryness using rotary evaporator at an optimium temperature of between 40 and 45 oC to avoid denaturation of active phyto-components.The extract was refrigerated at 2 ^{0}C – 8 ^{0}C.

The methanol and n-hexane extracts of the two plants were obtained by Soxhlet extraction. A 300g of C. filiformis (Pulverised) was extracted with 1.5 L methanol while A 400 g of *cassytha filiformis* was extracted with 1.5 L n-hexane.

Preparation and sterilization of media

All the media were prepared according to manufacturers instructions.

MICROBIOLOGICAL EVALUATION:

Isolation and characterization of test micro-organism

Clinical isolates *of E .coli, Pseudomonas aeruginosa, Proteus mirabilis,* and *Klebsiella spp* were obtained from patients visiting Adonai Diagnostic and Research Lab and Laboratory Department of Bishop Shanahan Hospital, both at Nsukka, Enugu State, Nigeria. The consent of the patients was gotten before sample collection .

Strains of test bacteria were isolated from samples of urine, high vaginal swab, urethral swab, endocervical swab and semen of patient manifesting symptoms of genito-urinary tract infections (Arora, 1999). The samples were immediately inoculated on blood agar plates and MacConkey agar plates. These plates were incubated a 37^{0}C for 24 h according to the method described by Cheesbrough (2000). Colonies suspected to belong to enterobacteriaecea were re-innoculated into slants of Kliglier iron agar (KIA) and incubated aerobically at 37 ^{0}C for a further 24 h for double sugar fermentation and hydrogen sulphide production. After the first 24 h, the bacterial colonies from cultures indicating infection or significant bacteriuria (Kass, 1956) were isolated, gram-stained and examined microscopically. All such isolates were also subjected to the following standard biochemical tests for micrococci and enterobacteriaecae: Catalase test, Coagulase test, Nitrate test, Indole, Use of KIA (for double sugar fermentation and H_2S production) (Cowan and Steel 1993: Cheesbrough, 2000) (Arora and Chugh, 1977).

Maintenance and standardization of stock cultures.

The stock culture of each clinical isolate was stored in nutrient agar slants (for bacteria) and SDA Slants (for fungi) at 4 ^{0}C. Prior to use, the cultures were activated by successive daily sub-culturing first into Blood agar and MacConkey agar plates and then into nutrient agar slant (for bacteria) (to ensure there was no contaminant) and SDA plates before sub culturing into SDA Slant (for *Candida sp*) for a period of 3 days.

The standardization of innoculum was carried out according to the method described by Arora (1999). The tops of 5-10 similar appearing, well isolated colonies on an agar plate were touched with a sterilized straight wire and then, inoculated in a nutrient broth medium. These broth bottles were incubated at 37 ^{0}C for 4 – 6 h to obtain the growth at logarithmic phase. The density of the organisms (bacteria) was adjusted to approximately 10^8 colony – forming units (CFU)/mL by comparing its turbidity with that of 0.5 McFarland opacity standards.

Preparation of turbidity standard equivalent to O.5 McFarland

The standard was prepared by using the techniques described by Cheesbrough (2000): A 1 % v/v solution of sulphuric acid was prepared by adding 1ml of concentrated H2S04 to 99 ml of water. A 1% w/v solution of barium chloride was prepared by dissolving 0.5 g of dihydrate barium chloride in 50 ml of distilled water. Then, 0.6ml of the 1% barium chloride solution was mixed with 99.4 ml of the 1% H_2SO_4.

A small volume of this turbid solution (barium sulphate) was transferred to a capped tube of the same type as the tubes used for preparing the test organisms

Preliminary sensitivity test

Preliminary antimicrobial screening of the extracts of both plants and standard antibiotic against the bacteria was done by the method of the cup-plate agar diffusion (Mirjana et al (1979), Boakye – Yiado 1979).

A 120 mg/ml of each of the extracts in DMSO was further two fold-diluted serially with sterile distilled water. Molten nutrient agar (20 ml each) were seeded with 0.1 ml of standardized broth cultures of bacteria . A total of 6 wells, 8 mm in diameter were made in the agar using a sterile cork borer. 0.06ml each of the two-fold dilutions was added into each labelled hole using a sterile pipette. As a control, 0.06ml DMSO was put in the centre well. Similarly, 2 fold dilutions of 0.120 mg/ml of Gentamicin (Gentalek) were added into respective agar-wells for comparison. The plates were left for 1hour at room temperature for diffusion after which they were incubated at 37^{0}C for 24 h for bacteria. Diameters of the zones of inhibition (IZD) were measured at the end of the incubation period. The mean of triplicate determinations was taken.

Evaluation of minimum inhibitory concentration (MIC) of extracts and the standard antibiotics.

The MIC of the antimicrobial agents were determined using agar dilution method (NCCLS, 1999).

Six (6) different concentration of each of the extracts in DMSO were prepared by two-fold dilution. The range of the concentrations of the extracts against test bacteria was 1.875 – 60 mg/ml. The antibiotic concentrations range from 0.0018– 0.060 μg/ml for Gentamicin.

With an automatic micropipette, 1.0 ml each of these different dilutions (one dilution per plate) of a single agent was introduced into individual agar plates. The molten agar, at 48 ^{0}C and the antimicrobial agents were mixed carefully and thoroughly and allowed to set. With the aid of a

sterile wire loop, the standardized test micro organisms were delivered on the agar surface of the plates containing different concentrations of the agent. This was done by streaking (about six-eight different strains of the isolates per plate) on the surface of the set agar. These inoculated agar plates were incubated at 37 ^{0}C for 24 h . At the end of the incubations, the MICs were determined as the lowest concentration of the extracts and the antibiotics that allowed not more than two colony forming units (cfu) to grow in it (Baron and Finegold 1980).

RESULTS AND DISCUSSIONS

Extraction yield

The percentage yield of the extracts increased with increasing polarity of the solvent; highest yield was noted with hot water, followed by methanol and the least was n-hexane extract **(Table 1)**.

Table 1:Extraction yield

Plant	Part	Percentage yield of the extract (%)		
		Hot Water	Methanol	n-hexane
Cassytha filiformis linn	Aerial parts	12.5	5.8	1.2

Sources, isolation and characterization of test micro organisms.

A total of 44 clinical uro-genital Gram negative bacteria were selected and labeled accordingly. All the isolates chosen conformed to standard identification and biochemical tests for the test micro-organism.

Table 2:Isolates of urogenital Gram negative bacteria& their sources

Pathogens	Sources and Number of Strains Isolated					
	Urine	HVS	ECS	US	Semen	Total
Klebsiella spp	9	2	0	1	0	12
Pseudomonas aeruginosa	6	2	0	1	0	9
Proteus mirabilis	6	1	1	2	0	10
Escherichia coli	10	2	0	1	0	13
						44

KEY: HVS = High Vaginal Swab; ECS = Endo Cervical Swab;US = Urethral SwabS; Semen = Seminal fluid with sperm cells

The **Table 2** above shows that the greatest number of micro-organism isolated from urine samples was E.coli. It is understandable because these are the micro-organisms most isolated in urinary tract infections. (Arora, 1999, Enweani et al, 1987.)

Preliminary antimicrobial sensitivity tests

The results of sensitivity tests of the extracts (methanol, hot water and n-hexane) of the plant part and standard reference drug against various microorganisms are presented in **Table 1** to **Table 6**. Both the methanol and hot-water extracts of the *Cassytha filiformis Linn* were effective against, *Escherichia coli*, *Pseudomonas aeruginosa* but had no activity against *Proteus mirabilis* and *kleb spp*.

Table 3: The inhibition zone diameter (IZD) of different extracts of *Cassytha filiformis linn* and Gentamicin against nine strains of *Pseudomonas aeruginosa*

Concentrations (mg/ml)	*IZD(mm) Mean ± SEM*		
	AF	MF	Gentamicin[È]
60.00	15.33 ± 0.69[a]	14.00 ± 0.69[a]	21.44 ± 1.09[b]
30.00	13.00 ± 0.55[a]	10.33 ± 1.46[a]	17.44 ± 1.08[b]
15.00	9.78 ± 1.27[a]	3.67 ± 1.84[b]	13.33 ± 0.71[c]
7.50	2.22 ± 1.47[a]	0.00 ± 0.00[a]	7.11 ± 1.79[b]
3.75	0.00 ± 0.00	0.00 ± 0.00	1.11 ± 1.11
1.88	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Key: a, b and c = levels of significance. Different superscripts in a row indicate significant differences between the groups (p<0.05). ml; [È] = IZD values were obtained using conc of gentamicin in µ

In **Table 3**, there is no significant difference between the activities of the extracts against *Pseudomonas aeruginosa*. When compared with gentamicin, the activities of each of the extracts seem lower; however, the reverse may be the case when the pure compounds from these extracts are used.

Table 4: The inhibition zone diameter (IZD) of different extracts of *Cassytha filiformis linn* and Gentamicin against thirteen strains of *Escherichia coli*

Concentrations (mg/ml)	*IZD(mm) Mean ± SEM*		
	AF	MF	Gentamicin [È]
60.00	14.85 ± 0.50[b]	16.69 ± 0.56[a]	13.15 ± 0.50[c]
30.00	13.15 ± 0.34[a]	14.46 ± 0.33[a]	8.31 ± 1.35[b]
15.00	8.92 ± 1.11[a]	11.31 ± 0.31[a]	1.62 ± 1.10[b]
7.50	0.77 ± 0.77[b]	1.54 ± 1.04[bc]	0.00 ± 0.00[b]
3.75	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1.88	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Keys:
a, b and c = levels of significance; Different superscripts in a row indicate significant differences between the groups (p<0.05); [È] = IZD values were obtained using conc of gentamicin in µg/ml

In **Table 4**, high values of IZD results showed that the extracts are very effective against Escherichia Coli. Compared with the gentamicin, the extracts produced promising IZD values. Apart from MF and AF at 60 mg/dl, all the extracts at lower concentrations show no statistical significant difference. The difference in the activities of the pairs of the extracts at higher concentrations can be attributed to differences in the rate of diffusion of their constituents through the agar. This study shows that the constituents of these extracts encounter diffusion problems at higher concentration of the extracts.

Table 5: **The inhibition zone diameter (IZD) of different extracts of Cassytha filiformis and Gentamicin against twelve strains of Klebsiella spp.**

Concentrations (mg/ml)	IZD(mm) Mean ± SEM	
	MF,AF,NF	Gentamicin È
30.00	0.00 ± 0.00[a]	16.00 ± 1.22[b]
15.00	0.00 ± 0.00[a]	12.50 ± 0.36[b]
7.50	0.00 ± 0.00[a]	8.58 ± 1.17[b]
3.75	0.00 ± 0.00[a]	1.50 ± 1.01[b]
1.88	0.00 ± 0.00	0.00 ± 0.00

Keys:

a,and b =levels of significance. Different superscripts in a row indicate significant differences between the groups ($p<0.05$). È = IZD values were obtained using conc of gentamicin in µg/ml

Table 6. The inhibition zone diameter (IZD) of *Cassytha filiformis* and Gentamicin against ten strains of Proteus *mirabilis*

Concentrations (mg/ml)	IZD(mm) Mean ± SEM	
	MF,AF,NF.	Gentamicin È
30.	0.00 ± 0.00	16.10 ± 0.53[b]
15.	0.00 ± 0.00	12.60 ± 0.40[b]
7.5.	0.00 ± 0.00	4.10 ± 1.68[b]
3.75.	0.00 ± 0.00	0.00 ± 0.00
1.88.	0.00 ± 0.00	0.00 ± 0.00

Keys:

a,and b =levels of significance. Different superscripts in a row indicate significant differences between the Groups ($p<0.05$). È = IZD values were obtained using conc of gentamicin in µg/ml

In **Table 5** and **Table 6,** it is clear that the extracts of *cassytha filiformis Linn* have no activities against *Klebsiella sp* and *Proteus mirabilis* respectively.

Minimum Inhibitory Concentration (MIC) of the Extracts and Standard Reference Antibiotics.

The MIC values of the extracts of the plant part and the antibiotics are presented in **Table 7**

Table 7 .The minimum inhibitory concentration (MIC) of different extracts of *Cassytha filiformis linn and standard reference antibiotic gentamicin* against strains of test bacteria.

MIC (mg/ml)

Bacteria	Mean ± SEM			
	AF	MF	NF	Gentamicin
Pseudomonas. aeruginosa	10.83±1.32[b]	13.33± 1.10[bc]	-	0.01 ± 0.00[d]
Escherichia. Coli	21.35±2.38[ab]	14.42 ± 0.58[b]	-	0.07 ± 0.01[d]
Proteus. Mirabilis	-	-	-	0.04 ± 0 .00[d]
Klebbsiella sp	-	-	-	0.10 ± 0.01[d]

Key: a, b, c and d = levels of significance. Different superscripts in a row indicate significant differences between the groups ($p<0.05$).

Against *Pseudomonas aeruginosa, E. coli* and *staph aureus*, strains, there is no significant difference in antibacterial activities between the hot water (AF)and methanol (MF)extracts of

Cassytha filiformis as shown by their MIC values against the bacteria studied (**Table 7**). It may, therefore, be advisable to use hot-water for the extraction of this plant parts whenever, there is a need to use it in the treatment of genito-urinary infection caused by these bacteria.

From the **Table**, it can be deduced from the MIC values of the gentamicin and all the extracts that these extracts contain active antibacterial ingredients that may compete with gentamicin or are more potent than the gentamicin against clinical bacteria isolates studied. The MIC value of the gentamicin against *E. coli* strains (0.071 ± 0.01 mg/ml) is very high compared with the MIC values against *Pseudomonas aeruginosa* (0.01 ± .00 mg/ml). This suggests higher efficacy of gentamicin against *Ps. aeruginosa* than *E. coli.*

The growths of *proteus mirabilis* and *Klebsiella* spp were not inhibited, in vitro, by all the extracts of *Cassytha filiformis Linn,* and therefore, no MIC was recorded with these extracts against the two bacteria..

From the MIC values of all the extracts, one can say with certainly that these extracts (except n-hexane extract of *Cassytha filiformis*) have broad spectrum of activities against the test micro-organisms. The explanation may be as a result of many phytoconstituents- alkaloids, flavonoid ,steroids,terpenoids- possessed by this plants (Sharma et al 2009) . The results also support the report that micro-organisms vary widely in the degree of their susceptibility (Emeruwa, 1982).

From this study, it can be stated that both extracts contain promising antimicrobial agents which when isolated and further purified can replace these common antibiotics in use to combat against the fast emerging resistant urogenital pathogens.

CONCLUSION

The results of this experiment, therefore, support the claims of some traditional medicine practitioners for the use of this plant as remedies against genito-urinary infections especially those caused by Gram-negative bacteria.

REFERENCES

- Arora, D.R.and Chugh, T .D. (1977) Characterization of micrococcaceac in clinical isolates. Indian J med Res; 65: 503-508.
- Arora, D.R (1999) Textbook of Microbiology 2nd Edn, CBS Publishing Company, New Delhi. pp 332-406.
- Boaky-Yiadon, K. (1979). Antimicrobial activity of aqueous extracts of *Crytptolepsis sanguinolenta* (Lindl) Schlechter. Quart. J. Crude Drug Res. 17(2): 78-80.
- Baron, E.J. and Finegold, S.M. (1990). Diagonstic Microbiology 8th ed. The C.V mosby company, Washington D.C, pp. 171-194.
- Change,C.W., Ko,F.N., Su,M.J.,Wu, Y.C. and Teng,C.M.(1997). Pharmacological evaluation of Octeine, Isolated from *Cassytha filiformis*, as an alpha (1)- adrenoceptor antagonist in rat thoracic aorta. Japanese J. Pharm. 73: 207-214
- Chang F, Chao Y, Teng C and Wu C. M(1998) Chemical constituents of Cassytha filiformis. J. Nat. Prod., 61(7),863-866.
- Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries Part 2 Cambridge University Press, Dock House, cape town 8001, S/ Africa. pp157- 159
- CLSI (2007). Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Seventeenth Informational Supplement M100-S17, CLSI, Wayne, PA, USA.
- Cowan, S.I. and Steel, K.J. (1993). Cowan and Steel's Manual for the Identification of Medical Bacteria. Barrow, G.I. and Felthanm, R.K.A. (eds.) Univ. Press,Cambridge.
- Emeruwa, A.I. (1982). Antibacterial substance from *Carica papaya* fruit extracts J. Nat. prod. 45 (2): 123-127.
- Enweani, I. B., Ogbonna, C. I. C. and Kozak, W. (1987). The incidence of candidiasis amongst the asymptomatic female students of the University of Jos, Nigeria. Mycopathologia 99: 135-141.
- Hoet, S., Stivigny, C., Block,S., Opperdoes, F.,Colson, P. and Baldeyrou, B. (2004).Alkaloid from *Cassytha Filiformis* and related aporphines: Antitrypanosomal activity, Cytoxicity and Interaction with DNA and Topoisomerase. Planta Medica, 70, 407-413.
- Kass, E.H. (1956). Asymptomatic infection of the urinary tract. Trans Assoc Am Phys 69: 56-63.
- Kirtikar, K.R .and Basu ,B.D. (1991). Indian Medicinal Plants. 2nd ed. 3: pp 1741-2163.

- Mirjana, K., Marica, M.S., Marija, M. and Dusica, M. (1979). Antibacterial and antifungal activities of isatin N-mannich bases. J. Pharm. Sc. 68(4):459-462.
- National Committee for Clinical Laboratory Standards (NCCLS). (1999). Performance Standards for Antimicrobial Susceptibility Testing 9th ed. Information Supplement M100-Sq Wayne pa:
- Scot C. Nelson (2008). Plant disease PD-42: http://www. ctahr. hawaii. edu/free pubs. s20/8/2010
- Sharma, S. Hullatti, K.K, Prasanna, S.M, Kuppast, I.J, Sharma, P.(2009). Comparative study of *Cuscuta reflexa* and *Cassytha filiformis* for diuretic activity. Pharma Res ; 1: 327-30
- Wu, Yang-Chang, Chang F-R, Ya-Chief, C. and Che-ming, T. (1998). Antiplatelet and vasorelaxant action of aporphinoids from *Cassytha filiformis*, Phytochem. Res; 12(SI), 539-541

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