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Phytochemical and Antimicrobial Assessment of Five Medicinal Plants Found in Terai Region

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Abstract

Crude petroleum ether and methanol extracts of different parts of five medicinal plants collected from Terai region, namely, *Asparagus racemosus*, *Catharanthus roseus*, *Hedychium coronarium*, *Mimosa pudica* and *Terminalia chebula* were examined for their antimicrobial activity against different pathogenic microorganisms, *Staphylococcus aureus*, *Escherichia coli*, *Klebsella* spp., *Salmonella paratyphi*, *Bacillus subtilis*, *Aspergillus niger*, *Fusarium* spp. and *Penicillium* spp. From the obtained growth curves of methanol extract of *Terminalia chebula* and petroleum ether and methanol extracts of *Hedychium coronarium*, it can be said these extracts have relatively high bacteriostatic property than other plant extracts. Similarly, the zone of inhibitions observed during antifungal assay by methanol extract of *Terminalia chebula* against *Aspergillus niger*, *Fusarium* spp. and *Penicillium* spp. are comparable to that of Itraconazole (antifungal drug). It shows that *Terminalia chebula* has fungistatic property. Phytochemical screening revealed the presence of alkaloids, coumarins, flavonoids, terpenoids, steroids and glycosides.

Key words: *bacteriostatic, crude extracts, fungistatic, growth curves, thin layer chromatography*

Introduction

Plants produce a huge array of different types of chemicals. Humans exploit these chemicals for a huge range of applications such as for drugs, flavors, fragrances etc. Nature has a rich reservoir of plants from which such structurally diverse chemical compounds can be extracted. Such diverse compounds include terpenoids, steroids, fatty acid-derived substances, polypeptides, alkaloids, non-ribosomal polypeptides and enzyme cofactors which are classes of secondary metabolites. These secondary metabolites are synthesized during adaptation to environmental stress and they have been exploited to get useful medicines to fight against diseases (Gupta 1994). This research has focused on phytochemical and antimicrobial properties of five different medicinal plants chosen on the basis of their availability and their medicinal values are *Asparagus racemosus*, *Catharanthus roseus*, *Hedychium coronarium*, *Mimosa pudica* and *Terminalia chebula*. Which were collected from Barahathawa, Sarlahi.

A. racemosus (family Asparagaceae) is locally known as Satawar. It is a shurb distributed (100-2100m) east to west in Nepal having small leaves, linear with small stout spinous spur at the base. Its fruits are globose (3-5mm) in diameter and turn bright red when ripened. It is fairly common in wild and gardens. The extracts of different parts of it are used as tonic, galactogogue and appetizer. The other uses of plants are in diuretic, aphrodisiac, tuberculosis, cough, and diarrhea (Kirtikar & Basu 1975). The major components reported from rhizome are carbohydrates, cardioglycosides, saponins, oils, fats, steroids, sterols, flavonoids, tannins, phenolic compounds and amino acids (Tenmozhi *et al.* 2010).

C. roseus (family Apocynaceae) is commonly known as Sadabahar, Nayantara, Sadaphal, Ainshakti, Pillaganneru, etc. It is a herb distributed (150-1500m) east to west in Nepal with a height about 1m. The leaves are ovate in shape. Flowers are found in axillary clusters of 2-3; petals are white with pink color and are

found throughout the years. The fruits are follicular. The juice of the leaves is beneficial in wasp-stings and menorrhagia. The roots are used as tonic and the major components reported are triterpenoids, tannins and alkaloids (Nayak & Pereira 2006).

H. coronarium (family Zingiberaceae) is commonly known as Dudh Kevara, which it is perennial, (1–1.5m) tall rhizomatous herb distributed (1500-2100m) east to west in Nepal. The leaves are simple, alternate, sheathed base and flowers are pure white with fragrance. The rhizomes are bitter and the major components reported from them are starch, albumin, alkenes, organic acids, essential oils, resins and diterpenes (Nakatani *et al.* 1994). The rhizome extracts have shown anti-inflammatory, analgesic (Shrotriya *et al*. 2007), antihypertensive (Ribeiro *et al.* 1986), diuretic (Ribeiro *et al*. 1988), leishmanicidal and antimalarial activities (Valadeau *et al*. 2009).

M. pudica (family Fabaceae) is commonly known as Lajjawanti and Buharijhar. This under-shrub is distributed (200-1200m) east to west in Nepal and recognized by its prickly hairy stems and sensitive leaves. The leaves are pinnate with four pinnae arranged palmately. Flowers are globose heads with purplish-pink in colour. Fruits are globular in clusters. The leaves and roots are useful in diseases of kidneys, piles and fistula. The roots are used in the treatment of asthma, fever, cough, dysentery, vaginal and uterine ailments and the major components reported are alkaloids, flavonoids, glycosides, triterpenes and saponin (Parekh & Chanda 2007).

T. chebula (family Combretaceae) is commonly known as Harro. It is a moderate sized tree distributed (150-1100m) east to west in Nepal. Leaves are simple, opposite, stalked and elliptic oblong. Flowers are yellowish white in terminal spikes. Fruits are drupes, yellow to orange brown. The fruits are used as tonic, to enrich blood, against diseases of spleen, piles, cold and for strengthening brain, eyes and gums. The major components reported in fruit extracts are triterpenoids, coumarin, tannin and phenolic compounds (Racadio *et al.* 2008). The fruit extracts are used in the treatment of asthma, sore throat, vomiting, hiccough, bleeding, piles, diarrhoea, gout, heart and bladder diseases and have shown antioxidant properties (Cheng *et al.* 2003), anticancer agents (Husheem *et al*. 2002), antidiabetic (Kirtikar & Basu 1935) and antimicrobial activities (Kumar *et al.* 2009). The biologically active compounds of this plant from petroleum extract are glycosides whereas methanol extract showed the presence of alkaloids, tannins, flavonoids, steroids, saponins and carbohydrate (Jayalaxmi *et al.* 2011).

Methodology

Plant materials

Five different medicinal plants *A. racemosus, C. roseus, H. coronarium, M. pudica* and *T. chebula* were collected from Barahathawa, Sarlahi as they were locally and easily available in most of the parts of this village. Different parts of plants used for the phytochemical and antimicrobial analysis were: tubers (*A. racemosus*), whole parts (*C. roseus*), rhizomes (*H. coronarium*), whole parts (*M. pudica*) and fruits (*T. chebula*)

Plant extraction

The plant parts were air dried, chopped, grinned and extracted for several hours with petroleum ether and methanol (cold extraction) in order to obtain non-polar to polar portion from the plant materials. Organic solvents were removed separately from each of these extracts under reduced pressure using rotavapour. Yield of each extract was calculated by the following formula:

$$\text{Yield (\%)} = \frac{\text{Weight of extract}}{\text{Total weight of sample}} \times 100$$

Phytochemical screening

Phytochemical screening was done in order to find the presence of the active chemical constituents such as alkaloids, steroids, flavonoids, reducing sugars, tannins, etc. The crude petroleum ether and methanol extracts of the plants were analyzed using standard phytochemical methods (Harborne 1998, Aguinaldo *et al*. 2005)

Thin layer chromatography

Methanol and petroleum ether extracts of the test plants were checked on thin layer chromatography (TLC) using different solvent system of varying polarity: Petroleum ether/Ethyl acetate (95:5), Petroleum ether/ Ethyl acetate (90:10), Petroleum ether/Ethyl acetate (80:20), Chloroform/Methanol (95:5) and Chloroform/Methanol (90:10). TLC plates were then observed through UV, Iodine, 5% H_2SO_4, $FeCl_3$ and Dragendroff's tests.

Antimicrobial Assay

Agar- well diffusion method

The solvents free petroleum ether and methanol extracts of *A. racemosus, C. roseus, H. coronarium, M. pudica* and *T. chebula* were tested against different pathogenic bacteria and fungi strains such as *S. aureus, E. coli, Klebsella* spp., *S. paratyphi, B. subtilis, A. niger, Fusarium* spp. and *Penicillium* spp. obtained from the Microbiology Laboratory, White House Institute of Science and Technology, Khumaltar, Lalitpur. These bacterial strains are selected as representative organisms for pathogenic Gram positive and Gram negative bacteria. The fungal cultures were used as per availability and their pathogenic natures. The concentration used was 1mg/ml. Bacterial inoculums were prepared in nutrient broth by taking loop full of organisms and incubating them at 37°C for 24hr. Fungal inoculums were prepared in Potato Dextrose Broth by taking few spores and incubating them at 25°C for 72hr. The antimicrobial activity was assayed by measuring the diameter of the inhibition zone formed around the well. The effects of different extracts of all the samples on microorganisms were then studied and their zones of inhibitions were compared taking Streptomycin as standard for antibacterial assay and Itraconazole as standard for antifungal assay.

Microtitration method

Methanol and petroleum ether extracts of *A. racemosus, C. roseus, H. coronarium, M. pudica* and *T. chebula* were tested for their property to inhibit the growth pattern of pathogenic bacteria. Streptomycin stock (1mg/ml) was prepared in phosphate buffer saline (PBS) and filtered through 0.45µm syringe filter and stored at -20°C. Broth culture of bacterial strain (500µl) was transferred into 5ml of freshly prepared sterile brain heart infusion broth (BHIB) which was then dispensed into 96 well plates along with filter sterilized solution of the plants extracts with final volume of 200µl in each well. Absorbance was taken at 630nm using microplate reader and data were retrieved using a printer for 9 hours at an interval of 1hr. Freshly, prepared sterile broth was also dispensed in some wells as natural control.

Results and Discussion

Extraction yields

The yield (%) of extracts was between 0.22-14.92percent. It was higher for methanol extraction than petroleum ether extraction indicating the presence of more polar fraction (Table 1).

Table 1. Extraction values of five plants in different solvents

Plants	Solvents	Extract yield (%)
A. racemosus	Petroleum ether	0.28
	Methanol	5.83
C. roseus	Petroleum ether	0.38
	Methanol	2.89
H. coronarium	Petroleum ether	1.46
	Methanol	5.47
M. pudica	Petroleum ether	0.22
	Methanol	1.8
T. chebula	Petroleum ether	0.27
	Methanol	14.92

Phytochemical screening

Phytochemical screening of the crude petroleum ether and methanol extracts of the five plants revealed the presence of different bioactive compounds (Table 2). Alkaloids were detected in petroleum ether and methanol extracts of *H. coronarium, M. pudica* and *T. chebula* whereas reducing sugars were observed in all cases. Terpenoids and steroids were also present in most of the extracts.

Alkaloids have bio-medicinal properties and used as actual drugs in pharmaceutical industry (Cordell 1981). Terpenes are valued for the presence of aromatic fragnance (Turner 1970), essential oils (Kovat 1987), etc. There is evidence that some of the phytosterols are effective against cardiovascular disease (Kris-Etherton *et al*. 2002). Presence of saponin means the plant has the detergent properties and piscidal property (Hostettman & Marston 1995).

TLC analysis

The TLC behavior of different crude extracts in different solvent systems showed the presence of compounds of different polarity. TLC of petroleum ether extract of *H. coronarium* in the solvent system petroleum ether/ethyl acetate (90:10) showed the

presence of 2-3 spots of alkaloids after spraying of Dragendroff's reagent (orange color appearance) having Rf = 0.66, 0.3 & 0.2. Similarly the appearance of orange color after spraying with Dragendroff's reagent in extracts of *M. pudica* and *T. chebula* indicated the presence of alkaloids.

Table 2. Result of the phytochemical test

Phytochemicals	*A. racemosus*		*C. roseus*		*H. coronarium*		*M. pudica*		*T. chebula*	
	Pet	Met	Pet	Met	Pet	Met	Pet	Met	Pet	Met
Alkaloids	-	-	-	-	+	+	+	+	+	+
Glycosides	-	+	-	-	-	+	-	-	-	+
Terpenoids	+	+	+	+	-	-	+	-	+	+
Steroids	+	-	+	+	-	-	+	+	+	-
Flavonoids	-	-	-	-	-	-	-	+	-	-
Reducing sugars	+	+	+	+	+	+	+	+	+	+
Coumarins	-	-	-	-	-	-	-	+	+	+
Tannins	-	-	-	-	-	-	-	-	-	-
Saponins	-	+	-	-	+	-	-	-	+	+

Pet: Petroleum ether, Met: Methanol. (+) and (-) signs indicate the presence and absence of phytochemicals respectively.

The spots on TLC of *M. pudica* and *T. chebula* extracts gave intense violet color, orange color, brick red spot while heated after spraying with 5% sulphuric acid. These indicate the presence of different terpenoids in the extracts and also confirmed by Liebermann Burchard test.

Thus, TLC is applicable for the detection of different chemical compounds. It is also useful in the separation of the pure compound according to its Retention factor value (R_f).

Antimicrobial assay

Antibacterial properties

The zone of inhibitions shown by petroleum ether extract of *H. coronarium* and methanol extract of *T. chebula* against *B. subtilis*, *S. aureus*, *Klebsella* spp. and *S. paratyphi* were comparable to that of streptomycin. *E. coli* was found to be resistant to all the samples except methanol extract of *T. chebula*. *S. paratyphi* was sensitive to most of the extracts (Table 3). The alteration of growth patterns of tested micro-organisms, *E. coli*, *Klebsella* spp., *S. aureus*, *S.*

Table 3. Zone of inhibitions of petroleum ether and methanol extracts of different plants on different pathogenic microbes

Plant/ Standard	Extract	Zone of inhibition (mm)				
		Gram positive bacteria		Gram negative bactaria		
		B. subtilis	*S. aureus*	*E. coli*	*Klebsella* spp.	*S. paratyphi*
A. racemosus	Petroleum ether	8	-	-	8	-
	Methanol	13	-	-	8	-
C. roseus	Petroleum ether	12	-	-	-	15
	Methanol	14	-	-	12	12
H. coronarium	Petroleum ether	21	16	-	13	16
	Methanol	17	10	-	12	-
M. pudica	Petroleum ether	9	-	-	8	-
	Methanol	11	9	-	8	16
T. chebula	Petroleum ether	10	-	-	7	-
	Methanol	15	15	15	14	15
Streptomycin	-	29	12	-	16	12
Control	-	-	-	-	-	-

paratyphi and *B. subtilis* might be due to the bacteriostatic property of respective extracts incorporated with them. From the obtained growth curves of methanol extract of *T. chebula* and petroleum ether and methanol extracts of *H. coronarium* (Fig.1-Fig.8); it can be said that these extracts have relatively high bacteriostatic property than other extracts. The antimicrobial activity showed that methanol extract of *T. chebula* and petroleum ether and methanol extracts of *H. coronarium* have relatively high antibacterial property which may be due to the presence of bioactive compounds present in the extracts.

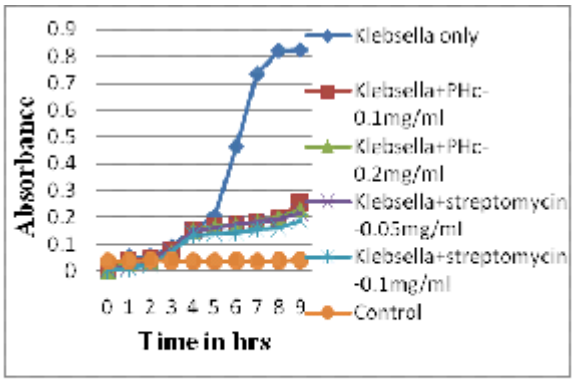

Fig. 1. Effect of *H. coronarium* petroleum ether extract on *Klebsella* spp.

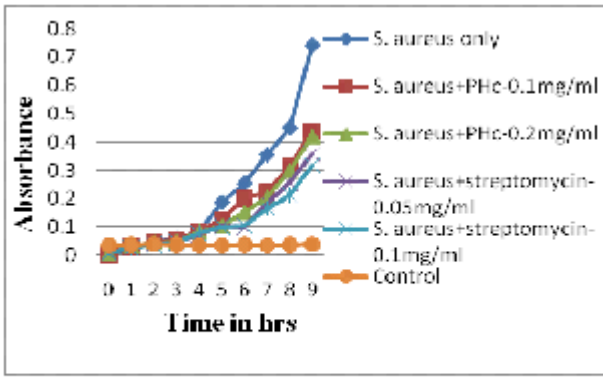

Fig. 2. Effect of *H. coronarium* petroleum ether extract on *S. aureus*

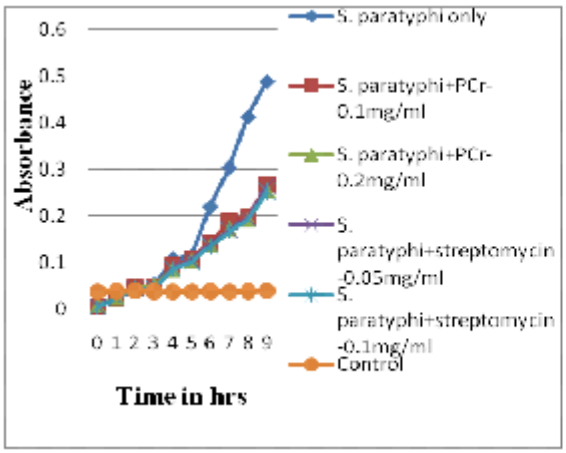

Fig. 3. Effect of *C. roseus* petroleum ether on *S. paratyphi*

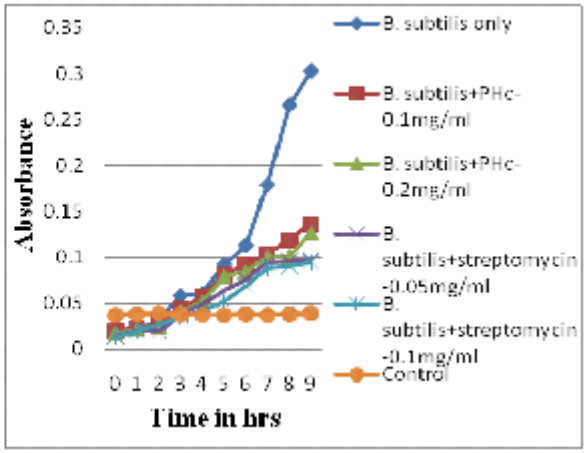

Fig. 4. Effect of *H. coronarium* petroleum ether extract on *B. subtilis*

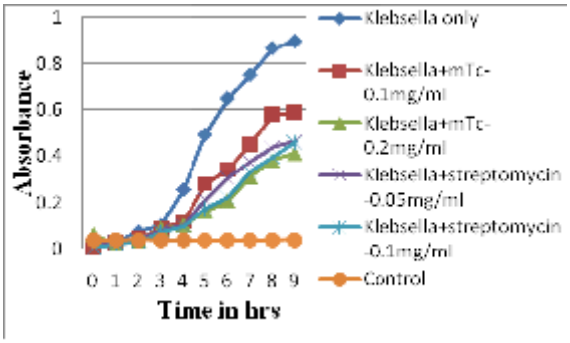

Fig. 5. Effect of *T. chebula* methanol extract on *E. coli*

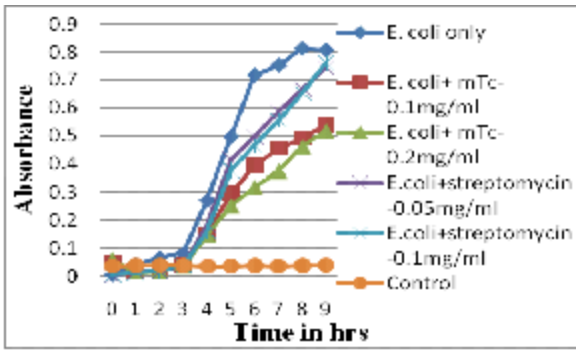

Fig. 6. Effect of *T. chebula* methanol extract on *Klebsella* spp.

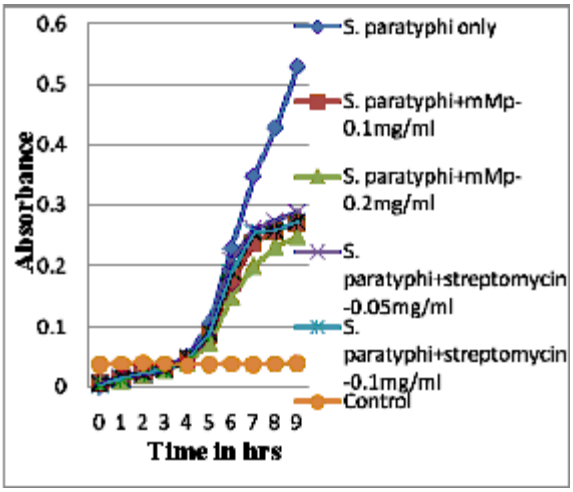

Fig. 7. Effect of *M. pudica* methanol extract on *S. paratyphi*

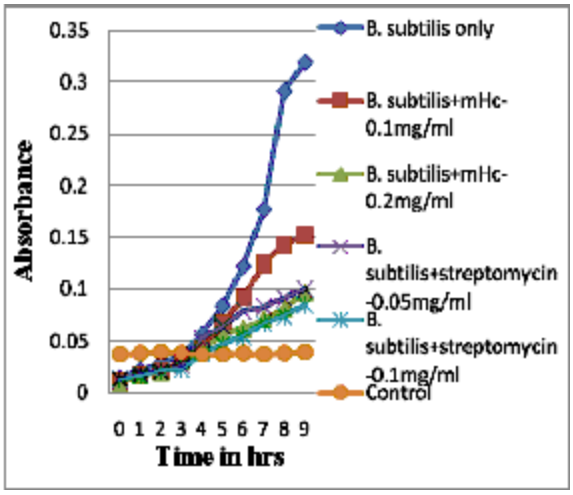

Fig. 8. Effect of *H. coronarium* methanol extract on *B. subtilis*

Antifungal properties

The zone of inhibitions (Table 4) shown by methanol extract of *T. chebula* against *A. niger*, *Fusarium* spp. and *Penicillium* spp. were comparable to that of Itraconazole (antifungal drug). It shows that *T. chebula* has fungistatic property due to the presence of bioactive compounds like: alkaloids, terpenoids and coumarins.

Table 4. Zone of inhibitions of petroleum ether and methanol extracts of different plants on different fungi

Plant/ Standard	Extract	Zone of inhibition (mm)		
		Aspergillus niger	*Fusarium* spp.	*Penicillium* spp.
A. Racemosus	Petroleum ether	-	-	-
	Methanol	-	-	-
C. roseus	Petroleum ether	-	-	-
	Methanol	-	-	-
H. coronarium	Petroleum ether	-	-	-
	Methanol	-	-	-
M. pudica	Petroleum ether	-	-	-
	Methanol	-	-	-
T. chebula	Petroleum ether	-	-	-
	Methanol	13	15	13
Itraconazole	-	13	-	-
Control	-	-	-	-

Different bioactive compounds such as, alkaloids, coumarins, flavonoids, terpenoids, steroids, reducing sugars, saponins and glycosides were successfully identified. Their antimicrobial activities were found satisfactory. However, the commercial production of these compounds is still far away. The main problem is due to the lack of optimization of cultural conditions and several strategies leading with increased accumulation of secondary metabolites. Detail studies are required to know the proper enzyme functions at various levels, product membrane permeability and adsorption for improvements towards achieving a viable economic production methodology. In addition, over-expression of enzymes and the genetic modification could be very useful via organogenesis or somatic embryogenesis for the production of desired levels of secondary metabolites.

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References

Aguinaldo, A.M., E.I. Espeso, B.Q. Guevara and M.G. Nonato. 2005. A guidebook to plant screening: Phytochemical and Biological (Ed. B.Q. Guevara). Manila University of Santo Thomas Press, The Philippines.

Cheng, H.Y., T.C. Lin, K.H. Yu, C.M. Yang and C.C. Linn. 2003. Antioxidant and free radical screening activities of *Terminalia chebula*. *Bio. Pharm. Bull*. **26**: 1331 1335.

Cordell, G. 1981. *Introduction to Alkaloids: A Biogenetic Approach*. Wiley and Sons, New York.

Gupta, S.S. 1994. Prospects and perspectives of natural plant products in medicine. *Ind. J. Pharmacol.* **26**.

Harborne, J.B. 1998. *Phytochemical methods*. Chapman and Hall, London. 7-8pp.

Hostettman, K. and A. Marston. 1995. *Saponins*. Cambridge University Press, United Kingdom.

Husheem, S.M., P. Harkonen and K. Pihlaja. 2002. Inhibition of cancer cell growth by crude extract and the phenolics of *Terminalia chebula* Retz. Fruit. *J. Ethnopharmacol.* **81**: 327336.

Jayalaxmi, B., K.A. Raveesha and K.N. Amruthesh. 2011. Phytochemical investigations and antibacterial activity of some medicinal plants against pathogenic bacteria. *J. Appl. Pharmaceut. Sci.* **2** (5): 124-128.

Kirtikar, K.R. and B.D. Basu. 1935. *Indian Med. Plant*. L.M. Basu, Allahabad. 1020 1023pp.

Kirtikar, K.R. and B.D. Basu. 1975. *Indian Med. Plants*. Dehradun, India. 2499-2500pp.

Kovat, E. 1987. Composition of essential oils, part 7. Bulgarian oil of rose (*Rosa damascena* Mill.). *J. Chromatogr.* A **406**: 185–222.

Kris-Etherton, P.M., K.D. Hecker, A. Bonanome, S.M. Coval, A.E. Binkoski, K.F. Hilpert, A.E. Griel and T.D. Etherton. 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am. J. Med.* **113** (Suppl 9B): 71S–88S.

Kumar, M., R.C. Agarawal, S. Dey, V.K. Rai and B. Johnson. 2009. Antimicrobial activity of aqueous extract of *Terminalia chebula* Retz. on gram-positive and gram-negative microorganisms. *Int'l. J. Curr. Pharmaceut. Res.* **1** (1): 56-60.

Nakatani, N., H. Kikuzaki, H. Yamaji, K. Yoshio, C. Kitora, K. Okada and W.G. Padolina. 1994. Labdane diterpenes from rhizomes of *Hedychium coronarium*. *Phytochemistry*. **37**: 1383-1388.

Nayak, B.S. and L.M.P. Pereira. 2006. *Catharanthus roseus* flower extract has wound-healing activity in Sprague Dawley rats. *BML Complementary and Alternative Medicine* **6**: 41.

Parekh, J. and S. Chanda. 2007. Antibacterial & Phytochemical Studies on Twelve Species of Indian Medicinal Plants. *African Journal of Biomedical Research* **10**: 175-181.

Racadio, S.P., G.V. Molina and R. Tacla. 2008. Phytochemical and microbial testing of Makahiya (*Mimosa pudica* Linn.) leaf extract. *UNP Res. J.* **XVII**: 11-18.

Ribeiro, R.A., M.M.F. DeMelo, F. DeBarros, C. Gomes and G. Trolin. 1986. Acute antihypertensive effect in conscious rats produced by some medicinal plants used in the state of São Paulo. *J. Ethnopharmacol*. **15** (3): 261-269.

Ribeiro, R.A., M.M.F. DeMelo, F. DeBarros, C. Muniz, S. Chieia, M.G. Wanderley, C. Gomes and G. Trolin. 1988. Acute diuretic effects in conscious rats produced by some medicinal plants used in the state of São Paulo. *J. Ethnopharmacol*. **24** (1): 19-29.

Shrotriya, S., M.S. Ali, A. Saha, S.C. Bachar and M.S. Islam. 2007. Anti-inflammatory and analgesic effects of *Hedychium coronarium* Koen. *Pak. J. Pharma. Sci*. **20** (1): 47-51.

Tenmozhi, M., R. Sivaraj and H.R. Yadav. 2010. A comparative phytochemical analysis of *Alstonia scholaris*, *Lawsonia inermis*, *Ervatamia divaricata* and *Asparagus racemosus*. *Int'l. J. Pharma.* **2**(9): 86-91.

Turner, A. 1970. Terpenoids and steroids. *Annu. Rep. Prog. Chem. Sect. B: Org. Chem*. **66**: 389–411.

Valadeau, C., A. Pabon, E. Deharo, J. Albán-Castillo, Y. Estevez, F.A. Lores, R. Rojas, D. Gamboa, M. Sauvain, D. Castillo and G. Bourdy. 2009. Medicinal plants from the Yanesha (Peru): Evaluation of the leishmanicidal and antimalarial activity of selected extracts. *J. Ethnopharmacol*. **123** (3): 413-422.