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Chemical Constituents of *Datura stramonium* L. Leaves and Its Antibacterial Activity against Human Pathogenic Bacteria

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ABSTRACT

The study of natural products has had a number of rewards. It has led to the discovery of a variety of useful drugs for the treatment of diverse ailments. The present study was phytochemical screening of the major secondary metabolites and evaluating their antibacterial activities of the crude extracts of *Datura stramonium* L. leaves. Extraction was done successively by maceration of leaf powder using petroleum ether, chloroform and methanol as solvents. Phytochemical screening was performed by various qualitative and quantitative methods. Antimicrobial activities of the crude extracts were determined by Agar well diffusion method against *Staphylococcus aureus, Shigella boydii, Streptococcus agalactiae* and *Klebsiella pneumonia*. The result of phytochemical analysis showed the presence of alkaloid, flavonoids, saponins, steroid, tannin and terpenoids and absence of quinones. Quantitative analysis of some of the detected phytochemicals showed saponins (44.61 mg/g) and alkaloids (39.10 mg/g) were dominant compounds followed by flavonoids (34.71 mg/g) and terpenoids (32.34 mg/g). Results of antibacterial assay revealed that extracts of the plant leaves showed inhibitory activity against the tested bacterial pathogens. Maximum inhibition was recorded against *Streptococcus agalactiae* (20.15±0.28 mm) and minimum inhibition against *S. aureus* (7.35±0.14 mm). Based on this results it is concluded that leaf extracts of the tested plant have the major secondary metabolites and antibacterial activities against the tested bacterial isolates.

Keywords: Chemical constituents, *Datura stramonium* L., Antibacterial Activity, Human pathogenic bacteria.

INTRODUCTION

Natural product chemistry is one of the most remarkable fields of study on health sciences; it is usually regarded by the layman as one of the most abstruse and remote from everyday life and thought. The term natural product is commonly reserved for those organic compounds of natural origin that are unique to one organism, or common to a small number of closely related organisms. The term secondary metabolite is typically used to refer to an organic compound of limited distribution in nature (Williams & Lemke, 2002).

The study of natural products has had a number of rewards. It has led to the discovery of a variety of useful drugs for the treatment of diverse ailments and contributed to the development of separation science and technology, spectroscopic methods of structure elucidation and synthetic methodologies that now make up the basics of analytical organic chemistry (Taura et al., 2014). Herbal remedies have been used for centuries but more recently the compounds that are active have been identified and this has enabled them to be extracted and purified. Synthetic organic chemists have then been able to produce the molecules *in vitro* and so produce them on larger scales.

In choosing medicinal plants for scientific evaluation of their biological activities and validation of ethnopharmacological usage criteria must be considered. Some criteria such as evidence of ethnopharmacological usage by the native population, the ailments which the plants are used to cure, the availability of the plant in its natural habitat, the sustainable use of the parts of the plant, and mode of preparation and administration by traditional healers have been reported (Suresh & Nagarajan, 2009).

Plant quality and pre-treatment are also important determinants of the phytochemical constituents and invariably the biological activities of an extract. These factors depend on plant parts used, genetic variation, geographical location, climatic conditions, collection period, drying methods, and storage conditions. Due to these possible variations, plant material from recognized botanical gardens or herbaria is usually recommended because they are protected, correctly identified and serve as reliable sources for

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subsequent collections. Preparation of voucher specimens is also an important aspect of medicinal plant research (Suresh & Nagarajan, 2009).

Datura stramonium L. is well known to have potent pharmacological activity with a great utility and usage in folklore medicine. The presence of different secondary metabolites has been reported previously in its leaf extracts (Sekhar et al., 2012). For example, tropane alkaloids such as scopolamine, hyoscyamine, and atropine are found in this plant, primarily in its seeds and flowers. Because of the presence of these fundamental phytochemicals, *Datura stramonium* L. is considered as treasured medicine and useful in the treatment of leukoderma, skin disorders, ulcers, bronchitis, jaundice, hysteria insanity, heart disease, fever and piles (Preissel & Preissel, 2002).

The determination of the phytochemical constituents of plant extracts is essential in order to ensure the reliability and repeatability of pharmacological and clinical research, and to understand their bioactivities and possible side effects of the active compounds through toxicity evaluation protocol. *Datura stramonium* L. is widely distributed in Ethiopia and known for its folk medicine including the treatment of bacteria. Thus, this study addresses on phytochemical analysis and evaluation of antibacterial potential of leave extracts of *Datura stramonium* L. against selected human pathogens.

MATERIALS AND METHODS

Materials and chemicals:

Grinder, rotary evaporator, digital measuring balance, shaker, Erlenmeyer flask, conical flask, Thin layer chromatography (TLC) plate (pre-coated aluminum sheet, 20 x 20 cm), UV lamp, water bath, test tubes, autoclave sterilizer, vortex, incubator, cork-borer, petri dish plates, different organic solvents and chemicals were used during this work. All test pathogens are obtained from school of veterinary medicine of Wollo University and clinically isolated.

Collection and preparation of plant material:

Mature leaves of *Datura stramonium* L. (local name: *Astenagir*, Amharic) used in this study were gathered from around Wollo University; Dessie Ethiopia (Fig. 1). The plant material was identified by Department of Biology, Wollo University, Dessie, Ethiopia. The leaves of *Datura stramonium* L. were washed thoroughly with distilled water. The leaves of *Datura stramonium* L. was subjected to drying at room temperature for about two weeks. The dried plant material was further crushed to fine powder using electronic grinder and the powdered sample was sealed in separate polyethylene bags until the time of extraction and further analysis.

Fig. 1: *Datura stramonium* L.
(Photograph, Mohammed A., 2019)

Preparation of the extracts:

The crude extracts of leaves of *Datura stramonium* L. were obtained using maceration method. The 400 g powdered sample was successively extracted with 2.0 L of petroleum ether, chloroform and methanol as the extraction solvents for 72 hours with occasional shaking (Abdullahi & Mainul, 2020). After extraction with each solvent the extracts were filtered using Whatmann filter paper to separate extract from the marc and then the removal of solvent was done under reduced pressure by Rotary evaporator. The masses of the extracts were weighted and stored at 5 ^{o}C for further analysis.

Analysis of phytochemicals:

Qualitative analysis of major secondary metabolites was carried out on the concentrated petroleum extract, chloroform extract, and methanol extract using standard procedures mentioned by different authors (Obdoni & Ochuko, 2001; Husain & Nagooru, 2011; Pralhad & Mishra, 2013; Malik & Ahmed, 2016). Leaf powder and preserved concentrated methanol extract of the test plant were used for standard quantitative estimation of the major secondary metabolites. The amount of total alkaloids (Gonzalez-Guevara et al, 2004), saponins (Gayathri & Kiruba, 2014), terpenoids (Gonzalez-Guevara et al, 2004) and flavonoids (El Bazaoui et al., 2011) were determined using standard protocols.

Thin Layer Chromatography analysis:

TLC Silica gel 60 F_{254} Multi-format pre-scored to 20 x 20 cm aluminum coated plates (stationary phase) were used. Two mobile phases standard consisted of a mixture of hexane and EtOAc for analysis of petroleum ether and chloroform extracts and one mobile phase consisted chloroform and ethyl acetate for methanol extract were prepared. TLC plate start lines were drawn (pencil) at 1.5 cm; giving an elution distance of maximal 4 cm. Spots were put on with a small capillary. UV

detection (254 nm and 360 nm) was carried out by UV light source. Chromatograms were further developed by vanillin/H_2SO_4 reagent. After the development of the chromatograms, the TLC plate was completely sprayed by a solution of this dyeing reagent and heated with a hot plate at 110 ^oC until the colored spots appeared. The spots were visually detectable after color development at room temperature.

Antibacterial assay:

Two gram-positive *(Staphylococcus aureus* and *Streptococcus agalactiae)* and two gram-negative *(Shigella boydii* and *Klebsiella pneumonia)* human pathogenic bacteria were considered. Agar well diffusion method was employed to assess the antibacterial activity of methanol, chloroform and petroleum ether extracts of tested plant against the human pathogenic bacteria. Selected enteric bacterial pathogens of human clinical isolates were obtained from Department of Veterinary medicine, Wollo University Dessie, Ethiopia.

Sub- culturing and standardization of inoculums:

Each of the enteric bacterial pathogen was cultured on separate Mulluer Hinton plate and incubated for 24 hours at 37 °C to obtain colonies. Two-three former colonies were picked up with a sterile inoculating loop and transferred into a test tube containing sterile normal saline solution and vortexed thoroughly. This was repeated until the turbidity of each bacterial suspension matched the turbidity of the 0.5 Mc Farland Standard (Dewick, 2004). The resulting suspension was then used as inoculum for the test pathogen used in the antibacterial susceptibility test.

Antibacterial activity test:

Preparation of test solution of extracts: The stock solution (0.5g/ml) was prepared by reconstituting 0.5 g of the dried extracts and dissolving in dimethyl sulphoxide (DMSO) until the volume of a solution became 1 mL. Different concentrations of extracts (200 mg/ml, 150 mg/ml and 100 mg/ml) were prepared after dilution of the stock solution with DMSO.

***Inoculation of Mueller Hinton Agar (MHA) plates*:**

A sterile cotton swab was dipped, rotated several times and pressed firmly on the inside wall of the tube above the fluid level within 15 minutes after adjusting the turbidity of the suspension of inoculums. This removes excess fluid from the swab. Then, the dried surface of MHA plate was inoculated by streaking the swab three times over the entire surface and rotating the MHA plates approximately 60°C each time to ensure an even distribution of inoculums.

Then, the MHA plates were left open for three to five minutes to allow for any excess surface moisture to be absorbed (Gayathri & Kiruba, 2014). Following this step,an equal distance holes with 6mm diameter were punched aseptically using flame sterilized cork borer tip. Prepared extract concentrations (50 µL) were introduced on the labeled wells using micropipette. The negative control (5% DMSO, 50µL) and positive control (Gentamicin, 10 µg) were placed into the labeled agar wells.

The plates were placed undisturbed at room temperature for 2 h and then incubated at 37℃ for 24 h. For each selected enteric bacterial isolate, the experiment was carried out in parallel and with three replications. Finally, the diameter of inhibition zone around the wells was measured in millimeter. The mean zone of inhibition and standard error of the mean (Mean± SEM) were calculated for the extracts and for standard positive control.

RESULTS

Determination of yield of extract:

The percentage yield of extracts from *Datura stramonium* L. leaf powder through successive solvent extraction (petroleum ether, chloroform and methanol) via maceration method were 5.96 % w/w, 16.31%w/w, and 19.85%w/w, respectively, Table 1.

Table 1: Successive Solvent Extraction of powdered *Datura stramonium* L. leaves

Solvents	Color of the extract	Yield (% w/w)
Petroleum ether	Yellowish green	5.96
Chloroform	Dark green	16.31
Methanol	Green and sticky with oil mass	19.85

Chromatographic analysis:

Petroleum ether, Chloroform, and Methanol extracts of *Datura stramonium* L. powdered leaf sample showed characteristic spots with the different solvent systems. Through several pilot experiments it was found that the compound fractions were separated by the solvent system of n-Hexane and ethyl acetate in the proportion of 8:2 and 7:3 for petroleum ether extract, n-Hexane and ethyl acetate (6:4) for chloroform extract and chloroform and ethyl acetate (9:1) for methanol extract (Fig. 2). The TLC chromatogram revealed 5 spots, 7spots and 9 spots for petroleum ether, chloroform and methanol extracts, respectively. Chloroform extract showed seven major spots with different Rf values 0.21(1), 0.31(2), 0.46(3),

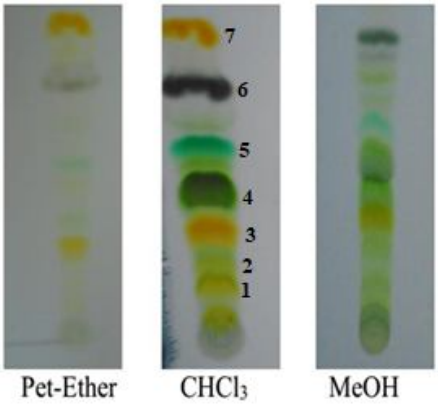

Fig. 2: TLC of crude extracts of *Datura stramonium* leaves (photograph)

constituents. The antibacterial activity of different solvent crude extracts of *Datura stramonium* L. leaves against *Staphylococcus aureus*, *Shigella boydii*, *Streptococcus agalactiae and Klebsiella pneumoniae* at concentrations of 100,150 and 200 mg/mL were evaluated and summarized in Table 4 and Fig 3.

DISCUSSION

The recovery of any particular bioactive compound fundamentally depends on the solvent of extraction and the plant part used for the extraction (Gul, 2017). The percentage yield results showed the yield varies with the type of solvent used. There was more yield of methanol than petroleum ether and chloroform. This suggests that the sample contain more polar bio molecules than nonpolar ones.

Table 2: Qualitative phytochemical analysis of crude extracts of *Datura stramonium* leaves

S. No	Secondary metabolites	Color observation	Types of extract		
			Pet-ether extract	$CHCl_3$ extract	MeOH extract
1	Flavonoid	Yellow	++	+	+
2	Tannins	Blue black	+	+	++
3	Saponins	Small bubble	-	+	+
4	Steroids	Red brown	+	+	+
5	Quinones	Red	-	-	-
6	Terpinoides	Radish brown	++	++	+
7	Alkaloids	Radish brown			
	• Mayer's test		+	+	++
	• Wagner's test		+	+	++

+ = detected ++ = strongly detected - = not detected

0.52(4), 0.70(5), 0.82(6) and 0.91(7). It showed that the presence of secondary metabolites like alkaloids those are indicative as yellow spot on TLC on vanillin reagent.

Phytochemical Analysis:

The qualitative phytochemical analysis of the major secondary metabolites was conducted for petroleum ether, chloroform and methanol extracts of *Datura stramonium* L. leaves. The characteristic colors were observed to confirm the presence of metabolites (Table 2). As shown in quantitative analysis there was significant difference between the different secondary compounds class in the tested plant. Saponins were the dominant compounds followed by alkaloids, flavonoids and terpenoids. The result showed that the amount of flavonoids, terpenoids, saponins and alkaloids present in methanol extract was detected as 34.41, 23.34, 44.61 and 39.10 mg/g (Fig. 2 and Table 3).

Antibacterial activity test:

The antimicrobial activity of a number of plants' extracts for the management and treatment of diseases is attributed to their phytochemical

Table 3: Quantitative analysis of phytochemicals form *Datura stramonium* leaves

Secondary metabolites	Amount of metabolites in mg/g
Flavonoids	34.71±0.005
Terpenoids	32.34±0.007
Saponins	44.61±0.007
Alkaloids	39.10±0.009

Measurement values are expressed as mean ± standard error of the mean (n=3)

TLC profiling of chloroform and methanol extracts gave an impressive result that directing towards the presence of number of phytochemicals. This variation in Rf values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. Here the different Rf values indicate the presence of different nature of phytoconstituents in single extracts.

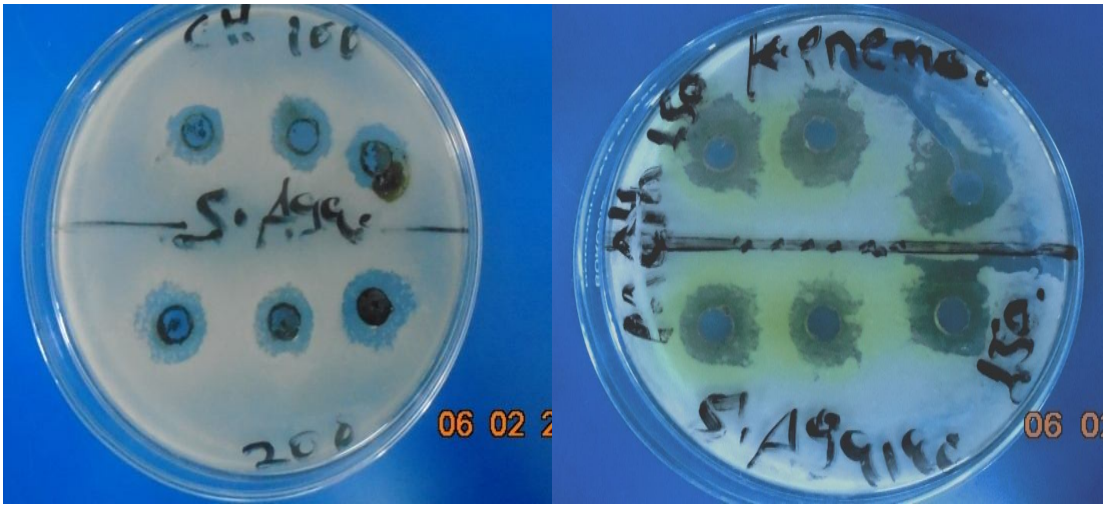

Fig. 3: Growth inhibition zone of extracts of *Datura stramonium* leaves (Photo: Mohammed)

Table 4: In-vitro antimicrobial activities of crude extracts of *Datura stramonium* leaves

Selected Bacteria	Conc. mg/ml	Types of crude extract			5% DMSO	10µg GEN.
		PECE	CCE	MCE		
S. aureus	100	7.35±0.14	7.72±0.06	10.60±0.60	0	24.37±0.01
	150	9.08±0.19	9.62±0.56	14.12±0.14		
	200	10.79±0.45	12.44±0.26	20.13±0.04		
S. agalactiae	100	7.78±0.13	10.07±0.13	13.58±0.72	0	23.62±0.01
	150	9.15±0.08	11.38±0.29	16.66±0.51		
	200	9.59±0.09	12.98±0.45	20.15±0.28		
K. pneumonia	100	8.99±0.10	7.91±0.17	8.58±0.74	0	13.01±0.04
	150	10.24±0.40	9.55±0.27	12.93±0.85		
	200	10.88±0.31	10.27±0.28	17.95±0.53		
S. boydii	100	9.67±0.12	8.46±0.13	8.25±0.42	0	20.98±0.14
	150	11.96±0.52	10.10±0.47	12.20±0.50		
	200	14.17±0.73	15.84±0.79	15.31±0.47		

GEN= Gentamicin, DMSO= dimethyl sulfoxide, PECE= Petroleum ether crude extract, CCE= Chloroform crude extract, MCE= Methanol crude extract and the inhibition zone were reported in mean (n=3) ± standard error of the mean for each triplicate data.

The results obtained from phytochemical analysis of this study pointed that the presences of flavonoids were confirmed by the yellow color observed due to test reagent. Alkaloids, tannins and steroids were detected as reddish brown color under respective test reagents. Moreover, saponins were characterized as blue black color. However, quinones were not detected in all of the extracts by the treatment of concentrated sulfuric acid.

The present study agrees with that of previous study which reported the presence of tannins, flavonoids, steroids and alkaloids in the leaf extracts of *Datura stramonium* L. using ethanol and methanol as solvents (Dai & Mumper, 2010). Using maceration method in ethanol and methanol another previous study by Baker, et al. (1995) also described the presence of tannins, steroids, flavonoids and alkaloids in the leaves of *Datura stramonium* L., however, terpenoids were not applicable. Preliminary phytochemical screening and in vitro antimicrobial activity of *Datura stramonium* L. leaves extract using maceration method was done by Anvir, et al. (2017). The author used chloroform, ethanol, hexane, petroleum ether and acetone as solvents and petroleum ether and chloroform leaf extract showed the presence of flavonoids, tannins, terpenoids and alkaloids which agrees with the present study. However, saponins were found in petroleum ether extract opposed to this study. The quantitative determination of chemical constituents of the plant studied showed that the leaves of *Datura stramonium* L. are rich in saponins alkaloids, terpenoids and flavonoids. This variation might be due the seasonal and geographical variation.

Phytochemical's constituents of plants are known to be biologically active compounds and they are responsible for different activities such as antioxidant, antimicrobial, antifungal and anticancer (Ananth, 2013; El Bazaoui et al., 2011). Previously many secondary metabolites have been

isolated and identified for their antimicrobial effect as mentioned Ajayi et al. (2011). These phytochemical substances including alkaloids, tannins, flavonoids and phenols have been known for their anti-diabetic, anti-atherosclerotic, anti-inflammatory, anti-carcinogenic and anti-microbial properties also reported by Baker, et al. (1995).

The presence of secondary compounds such as phenols, saponins, flavonoids, alkaloids, terpenoids, steroids and tannins are most likely to be responsible for the observed antibacterial activity. Secondary compounds exhibit their antimicrobial effect in different ways including inactivation of enzymes, cell envelope transport proteins and so forth (Adachukwu, et al, 2013). For instance, plant rich in tannins have antibacterial potential due to their character that allows them to react with proteins to form stable water-soluble compounds thereby killing the bacteria by directly damaging its cell membrane. Flavonoids are a major group of phenolic compounds reported for their antiviral, antimicrobial and spasmolytic properties. Alkaloids isolated from plant are commonly found to have antimicrobial properties. Pure isolated alkaloids and the synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and bacterial properties as per Biswas, et al. (2011). The presence of saponins supports the fact that *Datura* leaves have cytotoxic effects such as permealization of the intestine as saponins are cytotoxic.

Petroleum ether extract was produced maximum zone of inhibition against *S. boydii* at concentration of 200 mg/ml while minimum zone of inhibition against *S. aureus* at concentration of 100 mg/ml. Chloroform extract leaves of the plant revealed that maximum zone of inhibition against *S. boydii* at concentration of 200 mg/ml while minimum zone of inhibition against *S. aureus* at concentration of 100mg/ml. Methanol extract of the plant showed maximum zone of inhibition against *S. agalactiae* at concentration of 200 mg/ml while minimum zone of inhibition against *S. boydii* at concentration of 100 mg/ml .

The antimicrobial activities methanol and chloroform crude extracts of the plant were showed higher inhibition effect than petroleum ether extract against the tested bacteria strains. The crude extracts of the plant were indicated higher antibacterial activity with maximum zone of inhibition against *S. agalactiae* by methanol extract while minimum zone of inhibition against *S. aureus* by petroleum ether extract. Because the amount of active ingredients found in methanol extract was higher than petroleum extract due to its high polarity. In general methanol extract showed more activity efficacy against gram positive bacteria than gram-negative because those bacteria are less resistance due to lack of an outer membrane of the cell.

In conclusion, the results of qualitative phytochemical screening of leaves of the plant showed the presence of flavonoids, tannins, saponins, steroids, terpenoids and alkaloids whereas, quinones were absent in all extracts. Quantitative analysis showed that saponins and alkaloids are significantly higher than the rest of compounds in the plant leaves.

The result of anti-bacterial activity assay showed that extracts of tested plant leaves showed comparable with the positive control against all tested bacterial pathogens. Moreover, methanol extracts revealed promising activity than the pet ether and chloroform extracts. When the concentration extracts become 250 mg/ml, it showed greater efficacy than gentamicin in all cases. Therefore, it is concluded the tested plant leaves have the major secondary metabolites and antimicrobial property against the tested pathogens.

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COMPETING INTERESTS

The authors have declared that they have no competing interest.

REFERENCES

Abdullahi, R. A. & Mainul, H. (2020). Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *Journal of Pharmacy & Bioallied Sciences, 12*(1), 1–10.

Adachukwu, P., Ogbonna, A. O., & Eze, F.U. (2013). Phytochemical analysis of Paw-Paw (*Carica papaya*) leaves. *International Journal of Life Sciences Biotechnology and Pharma Research*, *2*(3), 347-351.

Ajayi, I. A., Ajibade, O.,&Oderinde, R. A. (2011). Preliminary Phytochemical Analysis of some Plant Seeds. *Research Journal of Chemical Sciences, 1*(3), 58-62.

Ananth, A. (2013). Phytochemical analysis of *Datura stramonium* L. as a potential medicinal tree:An overview. *International Journal of Pharmaceutical Science and Health Care, 5*(3), 2249-5738.

Anvir, A., Mizanur, S. K, &Shohael, A. A. M. (2017).Thin layer chromatographic profiling and phytochemical screening of six medicinal plants in Bangladesh *International Journal of Biosciences, 11*(1),131-140.

Baker,J.T., Boris, R. P., Carte, B., Cordell, G.A. & Soejarto, D. D. (1995). Natural product drug discovery and development: New perspectives on international collaboration. *Journal of Natural Products, 5,* 1325-1357.

Biswas, K. R., Khan, T., Monalisa, M. N., Swarna, A., Ishika, T.& Rahman, M. (2011). Medicinal Plants Used by Folk Medicinal Practitioners of Four Adjoining Villages of Narail and Jessore Districts, Bangladesh. *American- Eurasian Journal of Sustainable Agriculture, 5*(1), 23-33.

Dai, J. & Mumper, R.J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules, 15,*7313-7352.

Dewick, P. M. (2004).Medicinal Natural Products, A Biosynthetic Approach (2nd ed.). England, John Wiley and Sons Ltd, 164-170.

El Bazaoui, A., Bellimam, M. A. &Soulaymani, A. (2011). Nine new tropane alkaloids from *Datura stramonium* L. identified by GC/MS. *Journal of Fitoterapia*, *82*, 193–197.

Gayathri, V. &Kiruba, D. (2014). Preliminary phytochemical analysis of leaf powder extracts of *Psidium guajava* L. *International Journal of Pharmacognosy and Phytochemical Research, 6*(2), 332-334.

Gonzalez-Guevara, J. L., Gonzalez-Lavaut, J. A., Pino-Rodriguez, S., Garcia-Torres, M., Carballo-Gonzalez, M. T., Echemendiaarana, O. A., Molina-Torres, J. & Prieto- Gonzalez, S. (2004). Phytochemical screening and in vitro antitherapeutic activity of four Erythtroxylum species. *Acta Farmaceut Bonaer*, *23*(4), 506-509.

Gul, R., Jan, S. U., Faridullah, S., Sherani, S. & Jahan, S. (2017). Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from *Ephedra intermedia* Indigenous to Balochistan. *Scientific World Journal, 2017,* 5873648

Husain, M. A., & Nagooru, M. R. (2011). Biochemical profiling and total flavonoids contents of leaves crude extract of endemic medicinal plant *Corydyline terminals* L, Kunth. *Pharmacognosy Journal, 3*(24), 25-29.

Nagesh, M. &Samreen, A. (2016). Antimicrobial Activity of *Carica papaya, Piper nigrum* and *Datura stramonium* Plants on Drug Resistant Pathogens Isolated from Clinical Specimens. *IOSR Journal of Biotechnology and Biochemistry*, *2*(6), 57-59.

Obdoni, B. O. &Ochuko, P. O. (2001). Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure Applied Sciences., 8*(2), 203-208.

Pralhad, D. N.& Mishra, R. L. (2013). Quantitative Analysis of Secondary Metabolites of *Withania somniferaand Datura stramonium.International Journal of Science and Research,4,* 438-441.

Preissel, U. &Preissel, H. G. (2002). Brugmansia and Datura: Angel's Trumpets and Thorn Apples. Buffalo, NY: Firefly Books,106-129.

Sekhar, D., Kolanjinathan, K., Saranraj, P. &Gajendiran, K. (2012). Screening of *Phyllanthus amarus*, *Acalypha indica* and *Datura metal* for its antimicrobial activity against selected pathogens. *International Journal of Pharmaceutical & Biological Archives*, *3*(5), 1231-1235.

Suresh, S. N. & Nagarajan, N. (2009). Preliminary phytochemical and antimicrobial activity analysis of *Begonia malabarica* Lam. *Journal of Basic & Applied Biology*,*3*(1&2), 59-61.

Taura, D.W., Yushau, M., Bello, U. A., Hassan, A., Saidu, J.& Panda, T. W. (2014). Antibacterial activity of *Psidium guajava* in clinical isolates. *Academic Journal of Microbiology Research, 2*(2), 079-083.

Williams, A. D. & Lemke, L. T. (2002). Principle of Medicinal Chemistry (5th ed.). Philadelphia: Lippincott Williams and Wilkins, 24-25.