ISSN No:-2456-2165

# Comparative Study of a Proposed Green Extraction Method Named Aqueous Ultrasound Assisted Extraction from Fresh Leaves of *Acacia Nilotica* with Conventional Extraction Method

A F M Nazmus Sadat[1,2*], Mohammad Ali[2],Afroza Sultana[3], Muhammad Mehedi Hasan[2], Debobrata Sharma[2], Md. Atiqur Rahman[2] and Md. Abul Kalam Azad[1]

[1]Institute of Environmental Science, Rajshahi University, Rajshahi 6205, Bangladesh.
[2]Department of Pharmacy, University Of Development Alternative, Dhaka 1209, Bangladesh.
[3]Department of Nutrition and Food Technology, Jashore University of Science and Technology, Jashore-7408, Bangladesh.

**Abstract:- Aqueous extraction from fresh leaves of *Acacia nilotica* by Ultrasound Assisted Extraction (UAE)method was proposed in the present study. The intended method was occupying most of the features of the green extraction method. Efficiency and efficacy of the proposed extraction method was compared with the conventional extraction method and found satisfactory performance. The % yield (extraction efficiency) of the proposed method was observed higher than the conventional methanol and ethanol extraction. Similarly presences of tested phytochemicals werealso higher than the conventional method.Antimicrobial study (extraction efficacy) was performed against *S. aureus*(Gram +ve) and *S. dysenteriae* (Gram -ve) bacteria and found almost similar antimicrobial activities with the conventional crude extract. Based on the experiment the Aqueous UAE method may be standardized as an effective green extraction procedure.**

***Keywords:-*** *Green Extraction, Ultrasound Assisted Extraction, Staphylococcus Aureus, Shigella Dysenteriae.*

## I. INTRODUCTION

*Acacia nilotica* L. (Raheel *et al.*, 2014; Jamila and Rahman, 2016) commonly known as Babla in Bangladesh belonging to the family of Fabaceae (Raheel *et al.*, 2014; Rahmatullah *et al.*, 2009) is a commonly growing medium sized tree. *Acacia nilotica* popularly regarded as a medicinal plant in the South East Asian region (Munira*et al.*, 2013). From ethnobotanical survey it was observed that bark extracts is taken orally for curing bronchitis, whereas, pods extracts for dysentery and leaves for leucoderma by Santal tribe of Chapai Nobabgonj district in Bangladesh (Jamila and Rahman, 2016). Pods and tender leaves are also considered in folk medicine to treat diabetes mellitus (Ghani 1998). *A. nilotica* is a widely studied medicinal plant and reported various therapeutic uses including anti-asthamatic (Ali *et al.*, 2012), anti-bacterial (Abd*et al.*,1998; Sotohy*et al.*,1997; Ali *et al.*, 2012), anti-cancer (Malviya*et al.*, 2011), anti-diabetic (Ali *et al.*, 2012), anti-fungal (Ali *et al.*, 2012), anti-hypertensive properties (Gilani *et al.*, 1999; Ali *et al.*, 2012), anti-inflammatory (Dafallah and Al-Mustafa 1996), antioxidant (Malviya*et al.*, 2011), antipyretic (Ali *et al.*, 2012), antiscorbutic (Malviya*et al.*, 2011), antispasmodic (Gilani *et al.*, 1999; Ali *et al.*, 2012), astringent (Malviya*et al.*, 2011), bronchitis (Del 2009), congestion (Malviya*et al.*, 2011), diuretic (Malviya*et al.*, 2011), dysentery (Malviya*et al.*, 2011), hemorrhages (Malviya*et al.*, 2011), intestinal pains (Malviya*et al.*, 2011) and so on.

Ultrasound Assisted Extraction (UAE) process is a comparatively noble extraction method introduced end of the last century. Ultrasonic extraction is very useful for the isolation and purification of bioactive principles (Ishtiaq *et al.*, 2009). Application of high-intensity, high-frequency sound waves and their interaction with materials is the basic principle of extraction. Several probable mechanisms for ultrasonic enhancement of extraction including cell disruption, improved penetration and enhanced swelling, capillary effect, and hydration process have been proposed (Huaneng *et al.*, 2007). Previous studies observed that extraction variables of UAE method, particularly extraction time and temperature, strongly influence the extraction of phenolics, antioxidants, and anthocyanins (Ghafoor *et al.*, 2009). UAE gives the highest extraction yield of some flavonoids in lesser time in comparison to maceration and soxhlet extraction (Sun *et al.*, 2011). UAE is a potentially useful technology as it does not require complex instruments and is relatively low-cost.The method may be suitable both for small and large scale extraction (Dai and Mumper, 2010).

In the present study the Ultrasound Assisted Extraction method was optimized by using fresh leaves juice of *A. nilotica* instead of dried leaves powder to reduce the overall extraction process (Sadat *et al.*, 2019). Water is a universal solvent which is less hazardous and cheaper than the organic solvents. Theoretically all the compounds inside the cell was able to mixed with the surrounding deionized and slightly worm water during ultrasound treatment. The method successfully comply several points of "six principles of green extraction of natural products" (Chemat *et al.*, 2012) and may

be termed as a green extraction process. The objective of the present study was to standardizedthe use of optimized extraction procedure in comparison with the conventional extraction method.

## II. MATERIALS AND METHODS

### 2.1. Collection of Plant Material

The leaves of *Acacia nilotica* was collected from Botanical Pesticide Garden of the Institute of Environmental Science(IES) of Rajshahi University (RU), Bangladesh. The plant was identified by the professional taxonomist of the Department of Botany, RU and a voucher specimen was deposited at the herbarium of the institute.

### 2.2. Extraction Procedure

Fresh and shade dried leaves of *A. nilotica* was used in the study for comparison (Cheenickal and Mendez 2017). Fresh leaves were washed properly by running tap water followed by distilled water and placed in a shade for drying out the surface water (Francine *et al*., 2015). After 6 hours, 200gm fresh leaves were taken and divided into fourparts (Part A, B, C and D), 50gm in each. Part-A: fresh leaves were used immediately for UAE aqueous extraction and Part-B, Part-C and Part-D: fresh leaves were allowed for week long drying. After grinding 50gm fresh leaves of Part-B, Part-C and Part-Dreduced to 20.4gm, 19.7gmand 21.1gm respectively allowed for aqueous UAE, methanol and ethanol extraction.

Fresh leaves of Part-A of *A. nilotica* was blended in a conventional juice machine with 250 ml distilled water (material solvent ratio 1:5) for better extraction (Toma *et al.*, 2001). The juice was transferred to a 500ml conical flask and placed in an ultrasonic bath for 30 minutes treatments at 40°C bath temperature (Sadat *et al*., 2019). Fine powder of dried leaves (Part-B) was mixed with 102 ml distilled water (material solvent ratio 1:5) (Toma *et al.*, 2001) and treated in ultrasonic bath as like Part-A. Power Sonic 405 (Microprocess Controlled Bench Top Ultrasonic Cleaner) was used for sonication. The plant extract was then filtered through three layers of polyester cloth and dried at 60°C in a conventional water bath. Dried crude extract of aqueous UAEof Part-A and Part-B was thenstored in an air tight bottle labeled as E1 and E2 respectively and preserved in cold chamber for further use.

Fine powder of dried leaves of Part-C and Part-D were soaked in 98.5 ml of methanol and 105.5 ml of ethanol in a conical flask in a ratio 1:5 for 72 hours with intermittent shaking as per standard method (Hussain F and Hussain MM. 2012; Bashir *et al*., 2014; Latha *et al.*, 2015). The plant extract were then filtered by WhatmanNo.1 filter paper and concentrated by rotary evaporator under reduced pressure (in vacuum at 40°C). The dried methanol (Part-C) and ethanol (Part-D) extract was then stored in an air tight bottle labeled as E3 and E4 and preserved in cold chamber for further use.

### 2.3. Percentage of Yield Calculation

The percentage of yield indicate the efficiency of the extraction procedure which was calculated by using the following formula (Terblanche *et al*., 2017)

$$\%\, Yield = \frac{(W1\ x\ 100)}{W2} \text{---------- Eq. 1}$$

Where, $W1$ : weight of dried crude extract and $W2$ : weight of the plant starting material for extraction (Here, weight of fresh plant's was used for comparison).

### 2.4. Phytochemical Screening Test

Qualitative phytochemical test indicate the extraction efficiency of the crude extract. In the present study phytochemicaltests were performedin the following way;

**Alkaloids:** (I) Dragendoff's test: Mother solution + 2% of $H_2SO_4$+ Heat + few drops Dragendoff's reagent → Orange red precipitate [Trease and Evans, 1989; Ajayi and Fadeyi, 2015]; (II) Mayer's test: Mother solution + 2% of HCl + Heat + few drops Mayer's reagent → turbidity or yellow precipitation [Ajayi and Fadeyi, 2015; Dash *et al*., 2017]
**Anthraquinones:** Mother solution + benzene or chloroform + 10% (v/v) ammonia solution → pinkish or color change [Ayoola *et al*., 2008; Ajayi and Fadeyi, 2015]
**Flavonoids:** (I) Mother solution + dilute ammonia Solution + Conc. $H_2SO_4$ → yellow coloration that disappear on standing [Ayoola *et al*., 2008]; (II) Mother solution + few drops of 1% aluminium solution → yellow coloration [Ayoola *et al*., 2008]
**Glycosides:** Mother solution + 3 ml of glacial acetic acid + 1 drop of 5% ferric chloride Solution + 0.5 ml of Conc. $H_2SO_4$ →Brown or blue ring of the interface [Ayoola *et al*., 2008; Dash *et al*., 2017]
**Saponins:** Mother solution + equal volume water + vigorous shaken→ foam stable more than 10 minutes [Dash *et al*., 2017]
**Tannins:** Mother solution + 1% $FeCl_3$ solution → dark green color [Maxson and Rooney 1972; Hazali*et al*., 2015]
**Terpenoids:**Salkowski test: Mother solution+ 2ml Chloroform+ Carefully added 3ml Conc. $H_2SO_4$ to form a layer → reddish brown color of the interface [Ayoola *et al*., 2008]

### 2.5. Antimicrobial Activity

Antimicrobial study is a well-known in-vitro method for measuring efficacy (pharmacological activity) of the crude extracts and may be used for comparison of extraction procedures. Antimicrobial study was done by the disc diffusion method (Baker *et al*., 1993; Mukhtar and Tukur, 2000; Bauer *et al*., 1966; Servan*et al*., 2011; Latha *et al*., 2015) on*Staphylococcus aureus*(Gram +ve) and *Shigella dysenteriae* (Gram –ve).Microorganisms were collected from the Microbiology Lab, Department of Biochemistry and Molecular Biology, Rajshahi University, Bangladesh. Nutrient agar media was used for sub-culturing bacteria at 37°C. The filter paper discs (sensitivity discs) impregnated with the 300µg/disc of extracts was then placed on the surface of the inoculated nutrient agar with the aid of sterilized pair of forceps. A pre-diffusion time of 30 minutes

ISSN No:-2456-2165

was allowed for the extracts to diffuse from the discs into the agar medium before incubation. The degree of sensitivity of the organisms to the extracts was determined by measuring diameter of visible zones of inhibition to the nearest millimeter. The observed result of clear zone in petridish was compared to the standard zone of inhibition: <8 mm = no sensitivity; <10 mm= insignificant sensitivity; 10-15mm = moderately sensitive; >16mm = highly sensitive (Mukhtar and Okafor, 2002).

## III. RESULTS AND DISCUSSION

Crude extract obtained from aqueous UAE from fresh leaves of *A. nilotica* (E1)wasobserved dark-greenish incolor(Table-1) having yield value 14.8%(Table-2). Similar color was also observed in case of crude extracts obtained from E2 but the yield value (16.2%) was slightly higher than previous one. Methanol (E3) and ethanol (E4)crude extracts showed blackish in color having yield of 10.4% and 11.8% respectively. After conducting five successive extractions, a chart of extraction yield (Mean±S.D.) was prepared (Chart-1), which indicated that aqueous UAEextraction from fresh and dried leaves were better than conventional methanol and ethanol extraction and aqueous UAEextraction from fresh leaves was highest. Previous study observed that Jangade *et al.* (2014) found 9.48% yield from methanol extract whereas, Howlader *et al.* (2012) and Bashir *et al.*, (2014) found 13.26% and 10.4% respectively from ethanol extraction.The reference is almost similar to the present study. However, aqueous UAE method was observed better extraction than the conventional methanol and ethanol extraction procedure.

Dissolution studies indicated that crude extract of E1 was instantly soluble in water whereas freely soluble in methanol, ethanol and chloroform but sparingly soluble in DMSO, ethylacetate, dichloromethane (Table-3). Almost similar solubility profile was also observed in case of E2 crude extract. E3 and E4 crude extracts were observed instantly soluble in methanol and ethanol whereas freely soluble in water and sparingly soluble in DMSO, ethylacetate, dichloromethane. Solubility profile of E1 and E2 indicated that the crude extracts contain both polar (hydrophilic) and non-polar (lipophilic) compounds, which justify the use of UAE for extraction of wide range of compounds from the plant materials.

Table 1: Physical observation of the dried Crude Extract

| Crude Extracts | Optical Observation | Texture |
|---|---|---|
| E1 | Dark-greenish color | Hard and sticky to the beaker |
| E2 | Dark-greenish color | Hard and sticky to the beaker |
| E3 | Black | Soft and easy to withdraw |
| E4 | Black | Soft and easy to withdraw |

Here, E1: Aqueous UAE from Fresh leaves, E2: Aqueous UAE from dried leaves, E3: Methanol extract from dried leaves and E4: Ethanol extract from dried leaves

Table 2: Percent (%) Yield of Plants Extract by Aqueous UAE Method

| Extraction Method | Quantity of Plant Material for extraction | Quantity of Plant Material after drying | Solvent ratio | Quantity of Dried Crude extract | % Yield |
|---|---|---|---|---|---|
| E1 | 50 gm | - | 1:5 | 7.4 | 14.8 |
| E2 | 50 gm | 20.4 gm | 1:5 | 8.1 | 16.2 |
| E3 | 50 gm | 19.7 gm | 1:5 | 5.2 | 10.4 |
| E4 | 50 gm | 21.1 gm | 1:5 | 5.9 | 11.8 |

Here, E1: Aqueous UAE from Fresh leaves, E2: Aqueous UAE from dried leaves, E3: Methanol extract from dried leaves and E4: Ethanol extract from dried leaves

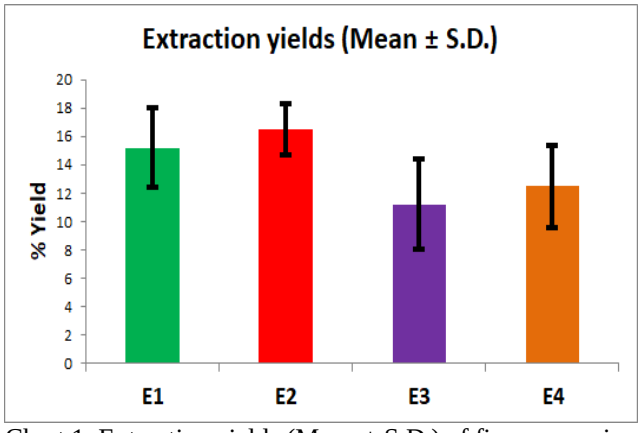


Chart 1: Extraction yields (Mean ± S.D.) of five successive batches

Table 3: Dissolution pattern of the dried crude extract

| Solvent | Crude Extracts | | | |
|---|---|---|---|---|
| | EI | E2 | E3 | E4 |
| Distilled water | +++ | +++ | ++ | ++ |
| Methanol | ++ | ++ | +++ | +++ |
| Ethanol | ++ | ++ | +++ | +++ |
| Chloroform | ++ | + | ++ | ++ |
| DMSO | + | + | ++ | ++ |
| Ethylacetate | + | + | + | + |
| Dichloromethane | + | + | + | + |

Here, E1: Aqueous UAE from Fresh leaves, E2: Aqueous UAE from dried leaves, E3: Methanol extract from dried leaves and E4: Ethanol extract from dried leaves

+++ Instantly soluble; ++ Freely soluble (1-3 minutes); + Sparingly soluble (> 5 minutes and vigorous shaking to dissolve)

ISSN No:-2456-2165

Phytochemical screening showed that crude extract from E1 and E2 of *Acacia nilotica* contain most of the tested compounds including alkaloid, anthraquinones, flavonoid, glycoside, steroid, tannin, terpenoid except saponin (Table-4). Observation also showed that anthraquinones, glycoside and saponin were absent in methanolic extract (E3) whereas anthraquinones, flavonoid and saponin were absent in ethanolic extract (E4). Previous study of Das *et al.* (2016) and Howlader *et al.* (2012) observed the absence of saponin and flavonoids respectively in their ethanol extract of *A. nilotica*. Similarly Jangade *et al.* (2014) reported the absence of saponin and anthraquinones in their methanol extract. Through phytochemical screening it was observed that ultrasound assisted extraction may be a suitable extraction procedure instead of conventional extraction procedure. Findings also indicated that drying stages of plants may be overlooked as similar compounds extracted both from the fresh and dried plant's parts during ultrasound treatment.

Crude extracts ofE1, E2, E3 andE4 of *A. nilotica* was proved promising antimicrobial agent observed from three successive antimicrobial studies on *S. aureus* and *S. dysenteriae*(Chart-2). Previous study of Howlader *et al.* (2012) and Bashir*et al.* (2014) observed significant sensitivity of ethanolic extract on *S. aureus,* whereas marked sensitivity was observed against *S. dysenteriae*by Das *et al.* (2016). Crude extract obtained from fresh leaves (E1) and dried leaves (E2) by UAE method showed almost similar antimicrobial activity. Statistically no significant difference was observed on the studied bacteria indicated the same efficacy of those two extract (Table-5).

Table 4: Phytochemical screening of aqueous UAE crude extract

| **Phytochemical Tests** | **Crude extract** | | | |
|---|---|---|---|---|
| | **E1** | **E2** | **E3** | **E4** |
| 1.Alkaloid,(i) Dragendroffs' test | + | + | + | + |
| (ii) Mayer's test | + | + | + | + |
| 2. Anthraquinones | - | - | - | - |
| 3. Flavonoid (i): by $H_2SO_4$ | + | + | + | - |
| (ii): by aluminum | + | + | - | - |
| 4. Glycoside | + | + | - | + |
| 5. Saponin | - | - | - | - |
| 6. Steroid | + | + | + | + |
| 7. Tannin | + | + | + | + |
| 8. Terpenoid | + | + | + | + |

Here, E1: Aqueous UAE from Fresh leaves, E2: Aqueous UAE from dried leaves, E3: Methanol extract from dried leaves and E4: Ethanol extract from dried leaves

Here, (+) indicated presence of compound, and (–) indicated absence of compound

Table 5: Antimicrobial activity of aqueous UAE crude extracts against bacteria

| **Pathogenic** | **Zone of Inhibition (mm) by using (300 µg/disc)*** | | | |
|---|---|---|---|---|
| | **E1** | **E2** | **E3** | **E4** |
| *S. aureus* (Gram +ve) | 18.00±3.61 | 19.33±2.52 (p=0.739) | 15.67±3.05 (p=0.465) | 16.67±3.06 (p=0.185) |
| *S. dysenteriae* (Gram -ve) | 15.67±2.52 | 16.67±3.51 (p=0.775) | 14.33±3.06 (p=0.578) | 15.00±3.61 (p=0.866) |

* Data was presented as Mean±S.D. considering 3 subsequent tests, p indicated difference with E1 (p>0.05, indicated difference was not significant).

Here, E1: Aqueous UAE from Fresh leaves, E2: Aqueous UAE from dried leaves, E3: Methanol extract from dried leaves andE4: Ethanol extract from dried leaves

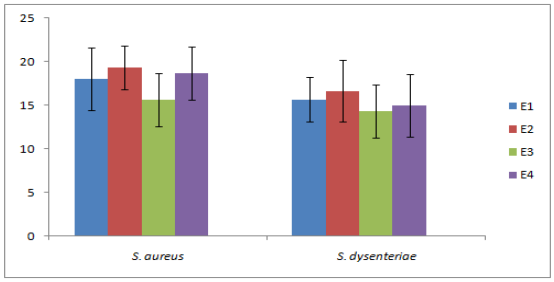


Chart 2: Comparison of antimicrobial study of the crude extracts of *A. nilotica* extracted by using different extraction method

(Here, E1: Aqueous UAE from Fresh leaves, E2: Aqueous UAE from dried leaves, E3: Methanol extract from dried leaves andE4: Ethanol extract from dried leaves)

## IV. CONCLUSION

The proposed "aqueous ultrasound assisted extraction from fresh leaves of *A. nilotica*" revels the similar efficiency compared with the conventional extraction method without compromising the efficacy of the crude extracts. Additionally this method successfully reduced the overall extraction process with simple instrument. The cost effective,

environment friendly, time efficient, operation simplicity nature of this method obeyed most of the principles of green extraction method. More study with different plant is recommended for establishing and popularizing this method in the extraction world of the natural products.

## REFERENCES

[1]. Ajayi AO and Fadeyi TE. 2015. Antimicrobial Activities and Phytochemical Analysis of Moringa oleifera Leaves on *Staphylococus aureus* and *Streptococcus species*. *American Journal of Phytomedicine and Clinical Therapeutics*, 3(10): 643-653.

[2]. Ali MS, Islam MS, Rahman MR, Sayeed MA and Islam MR. 2011. Antibacterial and cytotoxic activity of Ethanol Extract of *Mikania cordata* (Burm.F) B.L Robinson leaves. *Journal of Basic and Clinical Pharmacy*, 2(2): 103-107.

[3]. Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA ,Obaweya K, Ezennia EC and Atangbayila TO. 2008. Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research, September,*; 7 (3): 1019-1024.

[4]. Baker J, Liu JP, Robertson EJ and Efstratiadis A. 1993.Role of Insulin-like Growth Factors in Embryonic and Postnatal Growth.*Cell*, 75: 73-82.

[5]. Bashir H S, Mohammed AM, Magsoud AS, Shaoub AM. 2014. Isolation and Identification of Two Flavonoids from *Acacia nilotica* (Leguminosae) Leaves. *Journal of Forest Products & Industries*, 3(5), 211-215.

[6]. Bashir HS; Mohammed AM, Magsoud AS, Shaoub AM. 2014. Isolation and Identification of Two Flavonoids from *Acacia Nilotica* (Leguminosae) Leaves. *Journal of Forest Products & Industries*, 3(5): 211-215.

[7]. Bauer AW, Kirby WM, SherrisJC and Turck M. 1966.Antibiotic susceptibility testing by a standard single disk method.*Am J ClinPathol*, 45(4): 493-496.

[8]. Cheenickal M and Mendez RM. 2017.Phytochemical screening and the antimicrobial activity of the leaves of *Azadirachta indica*, A. Juss.*International Journal of Scientific & Engineering Research*, 8(5): 721-724.

[9]. Dafallah AA, Al-Mustafa Z. Investigation of the antiinflammatory activity of Acacia nilotica and Hibiscus sabdariffa, Am J Chin Med, 1996, 24(3-4), 263-269.

[10]. Dai J and Mumper RJ. 2010. Review Plant Phenolics: Extraction, Analysis and their Antioxidant and Anticancer Properties. *Molecules*, 15: 7313-7352.

[11]. Das M, Rahman MS, Maniruzzaman M and Uddin MB. 2016. Evaluation of Phytochemical, Invitro Antibacterial and Cytotoxic Properties of Ethanol Extract of *Acacia Nilotica* (L) Leaves. *Indo American Journal of Pharmaceutical Sciences*, 3(12): 1492-1497.

[12]. Dash SP, Dixit S and Sahoo S. 2017. Phytochemical and Biochemical Characterizations from Leaf Extracts from *Azadirachta Indica*: An Important Medicinal Plant. *Biochem Anal Biochem*, 6: 323. doi: 10.4172/2161-1009.1000323

[13]. Del WE. 2009. In Vitro evaluation of peroxyl radical scavenging capacity of water extract of *Acacia nilotica* (L).*Afr .Biotechnol*, 7: 1270-1272

[14]. Edeoga HO, Okwu DE and Mbaebie BO. 2005. Phytochemical constituentsofsome Nigerian medicinal Plants. *African Journal of Biotechnology*, 4(7): 685-686.

[15]. Francine U, Jeannette U and Pierre RJ. 2015. Assessment of antibacterial activity of Neem plant (*Azadirachta indica*) on *Staphylococcus aureus* and *Escherichia coli*. *Journal of Medicinal Plants Studies*, 3(4): 85-91.

[16]. Ghafoor K, Yong-hee C, Jeon J and Jo I. 2009.Optimization of Ultrasound-Assisted Extraction of Phenolic Compounds, Antioxidants, and Anthocyanins from Grape (*Vitis vinifera*) Seeds.*Article in Journal of Agricultural and Food Chemistry*, 57(11): 4988-94.

[17]. Ghani A. 1998. Medicinal plants of Bangladesh, Chemical constituents and uses, *Asiatic Press*, Dhaka : 1-33.

[18]. Gilani AH, Shaheen F, Zaman M, JanbazKH, Shah BH, Akhtar MS. 1999. Study on antihypertensive and antisplasmobic activity of methanol extracts of *Acacia nilotica* pods. *Phytother Res*, 13(8): 665-669.

[19]. Hazali NB, Ali MABM, Ibrahim MB and Masri M. 2015.Determination of phytochemicals and vitamin content of underutilized *Baccaureaangulata*fruit.*Journal of Pharmacognosy and Phytochemistry*, 4(4): 192-196

[20]. Howlader MSI, Hossain MH, Dey SK, Hira A, Ahmed A. 2012. Evaluation of antinociceptive, antimicrobial and cytotoxic activities of ethanolic extract of *Acacia nilotica* leaves from Bangladesh. *Pharmacology Online*, 2: 73-81.

[21]. Huaneng X, Yingxin Z and Chaohong H. 2007. Ultrasonically assisted extraction of isoflavones from stem of *Pueraria lobata* (Willd.) Ohwi and its mathematical model.*Chinese Journal of Chemical Engineering*, 15: 861-867.

[22]. Hussain F and Hussain MM. 2012.Cytotoxic Effect of Crude Extracts of Acacia Nilotica. International Journal of Pharmaceutical Sciences and Research, 3(6): 1652-1655.

[23]. Ishtiaq F, Farooq R, Farooq A, Siddique M, Shah H, Hassan MU, Shaheen MA. 2009. Application of ultrasound in pharmaceutics world. Appl. Sci. J. 6: 886-893.

[24]. Jamila M and Rahman AHM. 2016. Ethnobotanical Study of Traditional Medicinal Plants Used by the Santal Tribal Practitioners at the Village Jamtala of Chapai Nawabganj District, Bangladesh. *Journal of Progressive Research in Biology*, 3(1): 142-159.

[25]. Jangade NM, Nagargoje PB and Shirote PJ. 2014. Isolation, Phytochemical and Biological Evaluation of *Acacia nilotica* (L) Willd. Leaf Extract. *International Journal of Pharmacognosy and Phytochemical Research*, 6(2): 179-182.
[26]. Latha CR, Kavitha S and Sathya S. 2015. Antimicrobial Efficacy of *Azadirachta indica* Leaf Extracts. *International Journal of Science and Nature*, 6(3): 432-440.
[27]. Latha CR, Kavitha S and Sathya S. 2015. Antimicrobial Efficacy of *Azadirachta indica* Leaf Extracts. *International Journal of Science and Nature*, 6(3): 432-440.
[28]. Malviya S, Rawat S, Verma M and Kharia A. 2011. Preliminary Phytochemical Investigations of Acacia nilotica Linn Plant.*Current Pharma Research,* 1(2): 91-100.
[29]. Maxson ED and Rooney LW. 1972. Evaluation of methods for Tannin analysis in Sorghum Grain.American association of cereal Cherrism, inc., Minnesota 56121. 11pages.
[30]. Mukhtar MD and Tukur A. 2000.Antibacterial Activity of Aqueous and Ethanolic Extracts of *P. stratiotes*. *Journal of the Nigerian Society for Experimental Biology*, 1: 51-59
[31]. Munira S, Naim Z, Akter S , Majumder R, Kabir MH and Alam MB. 2013. Evaluation of Analgesic, Anti-Inflammatory and CNS Depression Activities of the Methanolic Extract of *Acacia nilotioca* Seed. *European Journal of Biological Sciences*, 5 (4): 123-130.
[32]. Raheel R, Aslam MS, Asghar S and Ashraf M. 2014.Phytochemical, Ethnopharmacological Review of *Acacia nilotica* (Desi Kikar) and Taxo-Pharmacology of Genus Acacia. *Indian Research Journal of Pharmacy and Science*, 1(2): 65-72.
[33]. Rahmatullah M, Mukti IJ, Haque AKMF, Mollik MAH, Parvin K, Jahan R, Chowdhury MH and Rahman T. 2009. An Ethnobotanical Survey and Pharmacological Evaluation of Medicinal Plants used by the Garo Tribal Community living in Netrakona district, Bangladesh. *Advances in Natural and Applied Sciences*, 3(3): 402-418.
[34]. Sadat AFMN, Mizan MRB, Sultana A, Rahman MM and Azad MAK. 2018. Comparative study of the Antimicrobial Activity of Methanol Extract and Ultrasound Assisted Water Extract of the Leaves of *Azadirachta indica*. *Rajshahi University Journal of Environmental Science*, 7: 40-47.
[35]. ServanES, Ionescu M, Matinca D, Maier CS and Bojita MT. 2011. Screening of the Antibacterial and Antifungal Activity of Eight Volatile Essential oils. *Farmacia*, 59(3): 440-446.
[36]. Sun Y, Liu Z and Wang J. 2011. Ultrasound-assisted extraction of five isoflavones from Iris tectorum Maxim.*Separation and Purification Technology*, 78(1): 49-54.
[37]. Toma M, Vinatoru M, Paniwnyk L and Mason TJ. 2001. Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. *Ultrason Sonochem*, 8: 137-142.
[38]. Trease GE and Evans WC. 1989. Pharmacology. 11th Edition.BailliereTindall Ltd., London, pp:60 – 75. 16. MaxsonE.D. and Rooney L.W. (1972).Evaluation of methods for Tannin analysis in Sorghum Grain.American association of cereal Cherrism, inc., Minnesota 56121. 11pages.