IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Green Synthesis of Copper Nanoparticles of Prunus domestica and its Evaluation of Anti Tubercular Activity

Prema. R[1*], Sharon. M. V[2], Senthil Kumar. C[1], Selva Bharathi. S[1], Prem Kumar. P[3], Sabitha. R[4] & Saratha. R[4]

[1]Sri Shanmugha College of Pharmacy, Sankari, Salem (Dist) - 637 304, Tamilnadu.

[2]Nehru College of Pharmacy, Thrissur - 680 588, Kerala.

[3]Tagore College of Pharmacy, Rathinamangalam, Chennai - 600127, Tamilnadu.

[4]School of Pharmacy, Dhanalakshmi Srinivasan University, Trichy- 621 112, Tamilnadu.

ABSTRACT:

One of the most recent areas of interest in the most recent nanotechnologies and nanoscience is the use of biomaterials in the synthesis of nanoparticles. More studies are being conducted on environmentally friendly methods of producing metal oxide nanoparticles (NP) in an effort to reduce the potential risks associated with dangerous substances in a secure setting. The safest technique for making copper nanoparticles is called green synthesis. Compared to other metal oxide nanoparticles (NPs), copper nanoparticles have a large surface area and are very biologically active. The plant that belongs to the Rosaceae family is called Prunus domestica. The majority of plant parts, including leaves, stems, flowers, and bark, have already been employed in the production of nanoparticles. To make Cu-NPs, fruit pulp extract is utilized. We discussed in this article how Prunus domestica aqueous extract is prepared to create copper nanoparticles (Cu-NPs) and how its phytochemical analysis is conducted. This article presents novel research that indicates a nano formulation produced from Prunus domestica has antitubercular potential. Our findings, substantiated through the Microplate Alamar Blue Assay (MABA) method, underscore the promising antitubercular activity of this innovative application, providing a potential avenue for the development of effective drug against tuberculosis.

Keywords: Prunus domestica, Green synthesis, Phytochemical analysis, Anti - tubercular activity, MABA method.

I. INTRODUCTION

The creation of nanoparticles with varying sizes, shapes, and chemical structures for a range of uses in human biology is the main focus of nanotechnology. With so many uses in electronics and medicine, nanotechnology is receiving more attention in the field of current research [1]. The production of nanoparticles (NPs) using plant extracts is currently in demand and is regarded as a bio-friendly technique for developing nanomaterials[2, 3]. Phytochemicals found in plant extracts, such as flavonoids, terpenoids, glycosides, alkaloids, and phenylpropanoids, were utilized in the biofabrication process to create nanoparticles. Plant extracts have the potential to be utilized in medical applications based on their latent biological properties, such as antimicrobial, antioxidant, antihyperglycemic, anti-inflammatory, or antimutagenic activity. These properties may also be demonstrated by the biotic features of the ensuing colloidal nanoparticle solution [4]. Due to their adaptability, metallic nanoparticles (NPs) have been widely used in a variety of industries and sectors, such as biosensors, catalysis, DNA analysis, cancer treatment, drug delivery, wastewater treatment, and solar power generation. It has been suggested that the environmentally safe and economical green synthesis of metallic nanoparticles is a viable substitute for physical and chemical methods. These days, a lot of study is focused on copper nanoparticles (Cu-NPs) because of their uses in industry and medicine[5]. As the name suggests, nanoparticles (NPs) are particles with a size range of 1–100 nm [6].The surface area to volume ratio of nanomaterials is significantly higher than that of their bulk counterparts, which can lead to more noteworthy catalytic reactivity and affect their quality [7]. Biomedical operations with metallic nanoparticles are incredibly promising and amazing. Silver, Aluminum, Gold, Zinc, Platinum, Titanium, Palladium, Iron, and Copper are the most commonly utilized metallic nanoparticles[8]. Scientists have been paying close attention to copper nanoparticles, or Cu-NPs, due to their prospective uses in modern medicine, as wound dressings, and because of their biocidal qualities[9]. Because dangerous, hazardous chemicals are used in some chemical processes to produce metal nanoparticles, these approaches are expensive and raise concerns about environmental hazards[10]. The creation of an environmentally friendly method has resulted from the application of green chemistry through biological procedure to synthesize necessary metal nanoparticles[11]. CuNPs' mechanical, optical, catalytic, and electrical qualities have garnered a lot of interest. Copper also helps in the creation of green nanoparticles. Copper is far more suited for a variety of uses due to its physical and chemical characteristics. Because they are affordable, they are also important in electronic circuits[12]. Due to their high surface to volume ratio, copper nanoparticles interact favorably with other particles. Over an extended duration, they function as antibacterial materials, extremely strong materials, sensors, catalysts, and sensors[13]. Cu-NPs have demonstrated noteworthy characteristics in the domains of optical devices, heat transfer nano-fluids, catalysts, and lubricants. Since they may be produced using a variety of techniques, copper nanoparticle production is fairly simple [14, 15, 16, 17]. These biological processes produced excellent yields at modest rates while reducing energy and cost at standard temperature and air pressure [18]. The creation of safe, clean, and ecologically acceptable green methods in place of traditional physical and chemical processes is one of nanotechnology's most important needs[19].

II. MATERIALS REQUIRED

Plum fruit, Copper sulphate solution, Beakers, Whatman filter papers

III. PREPARATION OF PRUNUS DOMESTICA (PLUM) EXTRACT

An appropriate number of fruits was carefully washed with distilled water to eliminate any impurities and dust. Fresh fruits of P-domestica (2000 g) were sliced and added in ethanol (70%) with a ratio of 1:3 w/v (P-dom: ethanol) at ambient temperature for 72 h under shaking at 300 rpm. The extracts were separated and filtered using a filter paper of Whatman No. 1. Then, the filtered extract was condensed by removing ethanol under reduced pressure at 40 °C, employing a rotary evaporator (100 rpm, low pressure) . The condensed and dried crude extract was accurately weighed and kept in a refrigerator at 4 °C for further studies [20].

IV. PRELIMINARY PHYTOCHEMICAL ANALYSIS[21 - 45]

Test	Procedure	Observations
Wagner's test	Few ml filtrate + 1 - 2 drops of Wagner's reagent (Along the sides of test tube)	_
Hager's test	Few ml filtrate + 1 - 2 ml Hager's reagents	_
Mayer's/ Bertrand's/ Valser's test A creamy	Few ml filtrate+ 1 - 2 drops of Mayer's reagent	_
Tannic acid test	Acidified extract + 10% tannic acid solution	_
Dragendroff's/ Kraut's test	Few ml filtrate + 1 - 2 ml Dragendroff's reagents	_
Bouchardat's test	6ml pulp extract, evaporated completely + 6mL ethanol (at 60 °C) + few drops of Bouchardat's reagent (dilute iodine solution)	_
Picric acid test	Few ml filtrate + 3 - 4 drops of 2% picric acid solution	_
sIodine Test	3ml extract solution + few drops of iodine solution	_
Test for starch	Aqueous extract + 5ml 5% KOH solution	_
Molish's test	2ml filtrate + 2 drops of alcoholic α–naphthol + 1ml Conc. H_2SO_4 (along the sides of test tube)	+
Barfoed's test	1ml filtrate + 1ml Barfoed's reagent + Heated for 2 min.	_
Seliwanoff's Test	1ml extract solution + 3ml seliwanoff's reagent + heated on water bath for 1 min.	_
Test for pentoses	2ml Conc. HCL + little amount of phloroglucinol + equal amount of aqueous extract solution + heated over flame	_
Resorcinol test	2ml aq. extract solution + few crystals of resorcinol + equal volume of Conc. HCL + heated	_
Fehling's test	1ml each of Fehling's solution A & B + 1ml filtrate + boiled in water bath A	_
Benedict's test	0.5ml filtrate + 0.5ml Benedict's reagent + Boiled for 2 min.	_

Legal's test	Dissolve 50gm pulp extract in pyridine + Sodium nitroprusside + 10% Sodium hydroxide	_
Concentrate H_2SO_4test	5ml pulp extract + 2ml glacial acetic acid + a drop of 5% $FeCl_3$ + Conc. H_2SO_4	_
10% NaOH test	1ml dil.H_2SO_4 + 0.2ml extract + boiled for 15min. + allowed cooling + neutralize with 10% NaOH + 0.2ml Fehling's solution A & B	_
Borntrager's test	2ml filtrated hydrolysate + 3ml Chloroform + shaken well + chloroform layer is separated + 10% Ammonia solution	_
Modified Borntrager's test	Pulp extract + ferric chloride solution + boil for 5min. + cooled + equal volume of benzene + benzene layer is separated + Ammonia solution	_
Keller-Killani test	1ml filtrate + 1.5ml glacial acetic acid + 1 drop of 5% ferric chloride + Conc. H_2SO_4 (along the side of test tube)	_
Kedee's test A disappearing	4ml extract evaporated to dryness + 1 - 2 ml methanol + 1 - 2 ml alcoholic KOH + 3 - 4 drops of 1% alcoholic 3, 5- dinitrobenzene + heated	_
Bromine water test	Pulp extract + few ml of bromine water	_
Millon's test	2ml filtrate + few drops of Millon's reagent	_
Xanthoproteic test	Pulp extract + Few drops of Conc. Nitric acid	_
Biuret test	2ml filtrate + 1 drop of 2% copper sulphate sol. + 1ml of 95% ethanol + KOH pellets	_
Ninhydrin test	2ml filtrate + 2 drops of Ninhydrin solution (10mg ninhydrin + 200ml acetone)	_
Shinoda's test/ Mg-hydrochloride reduction test	Pulp extract is dissolved in 5ml alcohol + Fragments of magnesium ribbon + few drops of Conc. HCL	+
Ferric chloride test	Extract aqueous solution + few drops 10% ferric chloride solution	+
Lead acetate test	1ml pulp extract + few drops of 10% lead acetate solution	+
Conc. H_2SO_4 test	Pulp extract + Conc. H_2SO_4	+

Shibata's reaction/ Cyanidin test	1gm Aq. extract + dissolved in 1-2 ml 50% methanol by heating + metal magnesium + 5 - 6 drops of Conc. HCl	+
Lead acetate test	Pulp extract is dissolved in 5ml distilled water + 3ml of 10% lead acetate solution	+
Ferric chloride test	Extract aqueous solution + few drops 5% ferric chloride solution.	+
Test for Cartenoids	(1gm extract + 10ml chloroform, vigorously shaken and filtered). Filtrate + Conc. H_2SO_4	+
Mitchell's test	Extract solution + iron + sodium tartarate (+ ammonium acetate solution)	_
Gelatin test	Pulp extract is dissolved in 5ml distilled water + 1% gelatin solution + 10% NaCl	_
10% NaOH test	0.4ml pulp extract + 4ml 10% NaOH + shaken well	_
Bromine water test	10 ml of bromine water + 0.5gm pulp extract	_
Spot test/ Stain test	Little quantity of pulp extract is pressed in between to filter papers	_
Saponification test	Extract + few drops of 0.5N alcoholic KOH + A drop of phenolphthalein (Heated for 2hrs)	_
Acetic anhydride test	1ml pulp extract + Acetic anhydride solution + 1ml Conc. H_2SO_4	_
Borntrager's test	10ml 10% ammonia sol. + few ml filtrate (shaken vigorously for 30 sec.)	_
Ammonium hydroxide test	10mg extract is dissolved in isopropyl alcohol + a drop of Conc. ammonium hydroxide solution	_
HCl test	2ml pulp extract + 2ml 2N HCL (+ Few ml ammonia)	_
NaOH test	Pulp extract + 10% NaOH + Chloroform	_

V. PREPARATION OF COPPER NANOPARTICLES OF PRUNUS DOMESTICA

Copper Nanoparticles of *Prunus domestica* (Cu-NPsPD) dissolved with suitable organic solvent (water miscible)

↓

Polymer and suspending agent containing aqueous surfactant solution at desired proportion Cu-NPsPD dissolved with suitable organic solvent (water miscible)

↓

The organic solvent completely evaporated by using a magnetic stirrer

↓

Preservative to the above suspension

↓

Finally using ultrasonicator under various cycles with cooling circulation to form Cu-NPsPD

Prunus domestica

Fruit Pulp Extraction

Cu-NPsPD solution

Figure 1: Preparation of Copper Nanoparticles of Prunus domestica

VI. PROCEDURE FOR ANTI-TB ACTIVITY USING ALAMAR BLUE ASSAY (MABA)

The anti Mycobacterial activity of compounds were assessed against M. tuberculosis using microplate Alamar Blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200µl of sterile deionized water was added to all outer perimeter wellsof sterile 96 wells plate to minimized evaporation of medium in the test wellsduring incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for fivedays. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagentand 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink colorwas scored as growth. The MIC was defined as lowest drug concentration which prevented the colorchange from blue to pink. [47]

Standard Strain used: Mycobacteria tuberculosis (Vaccine strain, H37 RV strain): ATCC No - 27294. Standard values for the Anti-TB test which was performed.

Isoniazid - 1.6 µg/ml; Ethambutol - 1.6 µg/ml; Pyrazinamide - 3.125 µg/ml; Rifampicin - 0.8 µg/ml; Streptomycin - 0.8 µg/ml

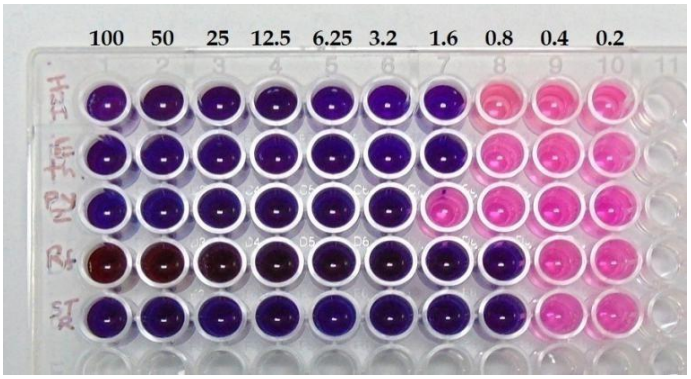

Figure 2: **Standard Drug Photograph**

VII. RESULTS

Sl. No.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.2 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
01	C1	S	S	S	S	R	R	R	R

Table 2: **Cu - NPsPD Concentration and its Sensitivity and Resistance**

Note:

S - Sensitive
R - Resistant

Figure 3: **Cu-NPsPD Sensitivity and Resistance**

VIII. CONCLUSION

This paper concentrated on the phytochemical study of fruit pulp extract and the green synthesis of Cu-NPs from the pulp extract of Prunus domestica. The plant Prunus domestica is a member of the Rosaceae family. A phytochemical investigation was done on fruit pulp. It demonstrates that the fruit pulp of Prunus domestica contains flavonoids, phenolic chemicals, and carbohydrates. Next, pulp extract from Prunus domestica was used to create copper nanoparticles. Thus, it can be said that environmentally beneficial and reasonably priced green synthesized copper nanoparticles made from extract from plum fruits. Our study on the nano formulation of Prunus domestica reveals significant antitubercular potential, validated through the Microplate Alamar Blue Assay (MABA). This breakthrough underscores the formulation's promising role in tuberculosis intervention.

REFERENCES:

[1] Narayanan, K.B. Sakthivel. Biological synthesis of metal nanoparticles by microbes. Adv Colloid Interface Sci. 2010; 156(1-2): 1 - 13.

[2] B. Moldovan, L. David, M. Achim, S. Clichici, and G. A. Filip.A green approach to phytomediated synthesis of silver nanoparticles using Sambucus nigra L. fruits extract and their anti-oxidant activity. J. Mol. Liquids. 2016; 221: 271 - 278.

[3] R. Sriranjani, B. Srinithya, V. Vellingiri, P. Brindha, S. P. Anthony, A. Sivasubramanian, and M. S. Muthuraman.Silver nanoparticle synthesis using Clerodendrum phlomidis leaf extract and preliminary investigation of its antioxidant and anticancer activities.J. Mol. Liquids. 2016; 220: 926 - 930.

[4] P. Orlowski, M. Zmigrodzka, E. Tomaszewska, K. RanoszekSoliwoda, M. Czupryn, M. Antos-Bielska, J. Szemraj, G. Celichowski, J. Grobelny, and M. Krzyzowska.Tannic acid-modified silver nanoparticles for wound healing: the importance of size. Int J Nanomedicine. 2018; 13: 991.

[5] K. Mittal, Y. Chisti, and U. C. Banerjee. Synthesis of metallic nanoparticles using plant extracts. Biotechnology Advances. 2013; 31(2): 346 - 356.

[6] B. Farias, A. F. Silva, R. Diniz Runo, J. Moura Luna, J. E. Gomes Souza and L. A. Sarubbo.Synthesis of silver nanoparticles using a biosurfactant produced in low-cost medium as stabilizing agent. Electronic Journal of Biotechnology. 2014; 17: 122.

[7] Y. L. Hewakuruppu.Plasmonic "pump–probe" method to study semi-transparent nanofluids. Applied Optics. 2013; 52(24): 6041 - 6050.

[8] P. Khanna, S. Gaikwad, P. Adhyapak, N. Singh and R. Marimuthu. Synthesis and Characterization Copper Nanoparticles. Materials Letters. 2007; 61: 4711 - 4714.

[9] M. Nasrollahzadeh, M. Maham, A. Rostami-Vartooni, M. Bagherzadeh and S. M. Sajadi. Barberry fruit extract assisted in situ green synthesis of Cu nanoparticles supported on a reduced graphene oxide–Fe_3O_4 nanocomposite as a magnetically separable and reusable catalyst for the O-arylation of phenols with aryl halides under ligand-free conditions. RSC Advances. 2015; 5(79): 64769 - 64780.

[10] Kathiresan, K. Manivannan, S. Nabeel, A.M. Dhivya. Studies on silver nanoparticles synthesized by a marine fungus, Penicillium fellutanum isolated from coastal mangrove sediment. Colloids Surf B Biointerfaces. 2009 June 1; 71(1): 133 - 137.

[11] Torresday, J.L.G. Parsons, J.G. Gomez, E. Peralta-Video, J. Troian, H.E. Santiago, Yacaman. M.J. Formation and Growth of Au Nanoparticles inside Live Alfalfa Plants. Nano Letters. 2002; 2(4): 397 - 401

[12] Schapter, A. K. Hu, H. Grenier, A. Schneider, R. Philips, F. Copper nanoparticles encapsulated in multi-shell carbon cages. Applied Physics.A. 2004; 78: 73 - 74.

[13] Narayanan R, El-Sayed M. A. Effect of Catalysis on the Stability of Metallic Nanoparticles: Suzuki Reaction Catalyzed by PVP-Palladium Nanoparticles. Journal of the American chemical Society. 2003; 125(27): 8340 - 8347.

[14] Nasibulin A. G, Ahonen. P. P, Richard. O, Kauppinen. E. I, Altman. I. S. Copper and copper oxide nanoparticle formation by chemical vapor nucleation from copper (II) acetylacetonate. Journal of Nanoparticle Research. 2001; 3: 383 - 398.

[15] Tatasov. S, Kolubaev. A, Belyaev S, Lerner. M, Tepper. F. Study of friction reduction by nanocopper additives to motor oil. Wear. 2002; 252(1): 63.

[16] Jeffrey A Eastman, S.U. S Choi, Shaoping Li, W. Yu, L. J.Thompson. Anomalously increased effective thermal conductivities of ethylene glycol-based nanofluids containing copper nanoparticles. Applied Physics Letters. 2001; 78: 718 - 720.

[17] Xiong Liu, W. Cai, Hui Bi. Optical absorption of copper nanoparticles dispersed within pores of monolithic mesoporous silica. Journal of Materials Research. 2002; 17: 1125 - 1128.

[18] Roza Bali, Nurhayati Razak, Ashok Kumar Lumb, Andrew. T. Harris. The Synthesis of Metallic Nanoparticles Inside Live Plants. IEEE Xplore Conference, Brisbane, Australia. 2006 July 3-7; 224 - 227.

[19] Zawrah M.F, Abd El-Moez. Antimicrobial Activities of Gold Nanoparticles against Major Foodborne Pathogens. Life Science Journal. 2011; 8: 37 - 44.

[20] Seraj Mohaghegh, Karim Osouli-Bostanabad, Hossein Nazemiyeh, Yousef Javadzadeh, Alireza Parvizpur, Mohammad Barzegar-Jalali, Khosro Adibkia. A comparative study of eco-friendly silver nanoparticles synthesis using Prunus domestica plum extract and sodium citrate reducing agents. Advanced Powder Technology.2020; 31: 1169 - 1180.

[21] Raaman, Nanjian. Phytochemical Techniques. New India Publishing Agency, New Delhi. 2006; 19 - 24.

[22] Singh V, Kumar R. Study of Phytochemical Analysis and Antioxidant Activity of Allium sativum of Bundelkhand Region. International Journal of Life Sciences Scientific Research. 2017; 3(6): 1451 - 1458.

[23] Silva GO, Abeysundara AT, Aponso MM. Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. American Journal of Essential Oils and Natural Products. 2017; 5(2): 29 - 32.

[24] Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Sciencia. 2011; 1(1): 98 - 106.

[25] Auwal MS, Saka S, Mairiga IA, Sanda KA, Shuaibu A and Ibrahim A. Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of Acacia nilotica (Thorn mimosa). Veterinary Research Forum. 2014; 5(2): 95 - 100.

[26] Ray S, Chatterjee S, Chakrabarti C.S. Antiproliferative Activity of Allelo chemicals Present in Aqueous Extract of Synedrella nodiflora (L.) Gaertn. In Apical Meristems and Wistar Rat Bone Marrow Cells. Iosr Journal of Pharmacy. 2013; 3(2): 1 - 10.

[27] Nanna RS, Banala M, Pamulaparthi A, Kurra A, Kagithoju S. Evaluation of Phytochemicals and Fluorescent Analysis of Seed and Leaf Extracts of Cajanus cajan L. International Journal of Pharmaceutical Sciences Review and Research. 2013; 22(1): 11 - 18.

[28] Kumar RS, Venkateshwar C, Samuel G, Rao SG. Phytochemical Screening of some compounds from plant leaf extracts of Holoptelea integrifolia (Planch.) and Celestrus emarginata (Grah.) used by Gondu tribes at Adilabad District, Andhrapradesh, India. International Journal of Engineering Science Invention. 2013; 2(8):65-70.

[29] Uma KS, Parthiban P, Kalpana S. Pharmacognostical and Preliminary Phytochemical Scr eening of Aavaarai Vidhai Chooranam. Asian Journal of Pharmaceutical and Clinical Research. 2017; 10(10): 111 - 116.

[30] Obouayeba AP, Diarrassouba M, Soumahin EF, Kouakou TH. Phytochemical Analysis, Purification and Identification of Hibiscus Anthocyanins. Journal of Pharmaceutical, Chemical and Biological Sciences. 2015; 3(2): 156 - 168**.**

[31] Deshpande PK, Gothalwal R, Pathak AK. Phytochemical Analysis and Evaluation of Antimalarial Activity of Azadirachta indica. The Pharma Innovation. 2014; 3(9): 12 - 16.

[32] Indhumati V, Perundevi S, Vinoja BN, Manivasagan, Saranya K, Ramesh, Babu NG. Phytochemical Screening and Antimicrobial Activity of Fresh and Shade Dried Leaves of Azaadirachta indica. International Journal of Innovations in Engineering and Technology. 2018; 11(3): 27 - 32.

[33] Bhatt S, Dhyani S. Preliminary Phytochemical Screening of Ailanthus excelsa Roxb. International Journal of Current Pharmaceutical Research. 2012; 4(1): 87 - 89.

[34] Basumatary AR. Preliminary Phytochemical Screening of some compounds from plant stem bark extracts of Tabernaemontana divaricata Linn. used by Bodo Community at Kokrajhar District, Assam, India. Archives of Applied Science Research. 2016; 8(8): 47 - 52.

[35] Audu SA, Mohammad I, Kaita HA. Phytochemical screening of the leaves of Lophira lanceolata (Ochanaceae). Life Science Journal. 2007; 4(4): 7 - 79.

[36] Sadasivam S, Manickam A. Biochemical methods. New Age International Limited, Publishers, New Delhi.2005; Edn 3: 1 - 4.

[37] Evans WC. Trease and Evans Pharmacognosy. Saunders Elsevier.2009; 16th Edn: 135 - 415.

[38] Sheel R, Nisha K, Kumar J. Preliminary Phytochemical Screening of Methanolic Extract of Clerodendron infortunatum. IOSR Journal of Applied Chemistry. 2014; 7(1):10 - 13.

[39] Pandey A and Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and Phytochemistry. 2014; 2(5):115 - 119.

[40] Jagessar RC. Phytochemical screening and chromatographic profile of the ethanolic and aqueous extract of Passiflora edulis and Vicia faba L. (Fabaceae). Journal of Pharmacognosy and Phytochemistry. 2017; 6(6): 171 - 172.

[41] Tyagi T. Phytochemical Screening of Active Metabolites Present in Eichhornia Crassipes (Mart.) Solms and Pistia stratiotes (L.): Role in Ethanomedicine. Asian Journal of Pharmaceutical Education and Research. 2017; 6(4): 40 - 56.

[42] Rahman MA, Rahman MA, Ahmed NU. Phytochemical and biological activities of ethanolic extract of C. hirsute leaves. Bangladesh Journal of Scientific and Industrial Research. 2013; 48(1): 43 - 50.

[43] Gul R, Jan SU, Syed F, Sherani F, Nusrat Jahan. Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from Ephedra intermedia Indigenous to Balochistan. The Scientific World Journal. 2017; 2017: 1 - 7.

[44] Njoku OV, Obi C. Phytochemical constituents of some selected medicinal plants. African Journal of Pure and Applied Chemistry. 2009; 3(11): 228 - 233.

[45] Maria R, Shirley M, Xavier C, Jaime S, David V, Rosa S et al. Preliminary phytochemical screening, total phenolic content and antibacterial activity of thirteen native species from Guayas province Ecuador. Journal of King Saud University Science. 2018; 30: 500 - 505.

[46] Kumar R, Sharma S, Devi L. Investigation of Total Phenolic, Flavonoid Contents and Antioxidant Activity from Extract of Azadirachta indica of Bundelkhand Region. International Journal of Life Sciences and Scientific Resesrch. 2018, 4(4): 1925 - 1933.

[47] Maria C. S. Lourenco, Marcus V. N deSouza, Alessandra C Pinheiro, Marcellede L. Fer reira, Rasnisb B, Goncalves, Thais Cristina M Nogneira, Monica A Peralta. Evaluation of anti-Tubercular activity of nicotinic and isoniazid analogues. ARKIVOC 2007; (xv): 181 - 191.