Herbal soap formulation using silver nanoparticles synthesized from bark of *Borassus flabellifer*

Divyabharathi * R, Palaniswamy * R

PG & Research Department of Biotechnology, Dr. N. G. P. Arts and Science College, Dr. N. G. P. Nagar – Kalapatti Main Road, Coimbatore- 641048. Tamil Nadu, India.

ABSTRACT:

Plant-based natural products have emerged as a desirable substitute in recent years for improving the significant biological properties of medicinal soaps. A plant from the Arecaceae family is *Borassus flabellifer Linn*, commonly known as palmyra palm. Bark of *Borassus flabellifer* is a source of several phytochemicals, such as polyphenols, Steroids, alkaloids, Terpenoids, Tannins, resins, proteins, carbohydrates, and saponins. These *Borassus flabellifer* bark are used to synthesis as silver nanoparticles, which are used to treat a variety of diseases, especially ones where the anti-inflammatory properties are needed. The bark of AgNPs can be examined using a UV-Visible Spectrophotometer, FE-SEM and FTIR, among other techniques, to determine their size, crystal structure, elemental composition, and other characteristics. Followed by the Physical, Chemical and Biological examination in the soap formulation incorporates these AgNPs, leveraging their antimicrobial property thus enhancing the efficacy of soap, hygiene, and promoting public health.

Keywords: *Borassus flabellifer*, phytochemical analysis, UV-Visible Spectrophotometer, FE-SEM and FTIR, Evaluation and efficacy of soap.

1. INTRODUCTION:

Many plants have been used as medicine since the beginning of human history. For both complete and partial plants and trees, numerous therapeutic applications have been discovered [1]. *Borassus flabellifer*, commonly known as the (palmyra palm or toddy palm), is a versatile plant renowned for its various applications [2]. Its bark potential in producing silver nanoparticles and eco-friendly properties [3]. *Borassus flabellifer* is a source of several phytochemicals and antimicrobial properties, making them valuable in medical and industrial settings [4]. *Borassus flabellifer* is a source of several phytochemicals, such as polyphenols, carotenoids, alkaloids, polysaccharides, steroidal glycosides, abuminoids, triterpene, and saponins [5]. These bioactive components gave the plant anti-inflammatory, antibacterial, antioxidant, antidiabetic, and antihyperlipidemic qualities [6] [7]. Many things, including microorganisms, food, cosmetics, medications, and the environment, can cause allergies [8]. The bark of the *Borassus flabellifer* plant produces silver nanoparticles that can be used to treat various disorders. In essence, silver nanoparticles have anti-microbial and anti-human pathogen properties [9]. Following the synthesis, the plant extract *B. flabellifer* was characterized by UV-Vis Spectrophotometer, FTIR, FE-SEM, TEM and XRD to determine which nanoparticles had the highest efficacy and effectiveness [10][11]. These nanoparticles can be produced for commercial use and a toxic-free environment by being applied as soap, oilments, wound sprays, and medications [12].

2. METHODOLOGY:

The synthesized herbal soap was evaluated by various analysis and screened the antimicrobial effect of herbal soap synthesized from *Borassus flabellifer.*

Sample collection and Preparation of Extract:

The fresh bark of *Borassus flabellifer* were obtained from manickampalayam village, Coimbatore district. Plant bark were thoroughly washed, air dried and powdered. A 10mg of plant powder or fresh bark were soaked in 100ml of distilled water and boiled at 60˚c for 20 to 25 minutes. It was filtered through Whatman No.1 filter paper. The colour, consistency and yield of extract were noted.

Phytochemical analysis:

Phytochemical analysis was done by using aqueous extract of bark sample from of *Borassus flabellifer.*

Test for Alkaloids:

2ml of extract was treated with few drops of Mayer's reagent. Presence of white creamy precipitate indicates the positive test.

Test for flavanoids:

2ml of extract was treated with few drops of 1N sodium hydroxide solution and observed the formation of intense yellow colour and this becomes colourless on addition of dilute hydrochloric acid, indicating the presence of flavonoids.

Test for steroids:

2ml of extract was dissolved in 10ml of chloroform. To this mixture equal volume of concentrated sulphuric acid was added by sides of the test tubes. The upper layer becomes red while lower layer of sulphuric acid turns yellow in colour with green fluorescence indicating the presence of steroids.

Test for Terpenoids:

2ml of extract was treated with 2ml of acetic anhydride. Few drops of concentrated sulphuric acids were then added to this solution and observed the formation of blue, green rings that indicates the presence of Terpenoids.

Test for saponins:

2ml of extract was taken in a test tube and 6ml distilled water was added to it. The mixture was then shaken vigorously. The persistence of foam was observed that indicates the presence of saponins.

Test for tannins:

2ml of extract was allowed to react with 10% alcoholic ferric chloride solution. Formation of blue or greenish colour indication of presence of the tannins.

Test for phenol:

Few drops of the extract were treated with 5% aqueous ferric chloride. Formation of deep blue or black colour indicates the presence of phenolic compounds.

Test for resins:

2ml of the extract were treated with few drops of sulphuric acid solution. The yellow colour ring indicates the presence of resins.

Test for proteins:

2ml of the extract were treated with 0.2% freshly prepared Ninhydrin solution. The red color indicates the formation of protein presence.

Test for carbohydrates:

2ml of the extract were treated with few drops of concentrated sulphuric acid. The violet colour indicates the presence of carbohydrates.

Synthesis of silver nanoparticles from bark sample:

1 mM (0.017g) silver nitrate solutions add into 100ml of distilled water. From this remove 10 ml of silver nitrate solution, add 80 ml of plant bark extract. Then check the pH value (7.4). In this mixture, the color changed from light brown to dark brown, indicating the formation of silver nanoparticles. The mixture was centrifuged, pellets collected and dried overnight in a hot air oven at 60˚C. a dark brown color powder obtained was stored at room temperature for further use.

Characterization of nanoparticle:

The extracted bark were characterized by UV Visible spectrophotometry used to find out the overall quantity. Here, in nanoparticles production their characterization like size, shape, and stability of nanoparticles. Fourier- Transform Infrared Spectroscopy is perhaps the most powerful tool for identifying the types of chemical bond/functional groups present in the phytochemicals and FE-SEM enables qualitative and quantitative characterization of nano particles and also it can reveal internal structures of nano particles.

Antimicrobial Activity:

The anti-microbial activity action of the aqueous bark extract was determined by obtaining clinical pathogens from KMCH, Coimbatore such as *Escherichia coli and P. aureus* were studied. The study was done by using Turbidity assay. Each conical flask mixed with *E.coli* and *P. aureus* culture. Control conical flask only having broth and standard having nutrient broth with isolated *E.coli* and *P. aureus* culture. Another hand, 5 different concentration of nanoparticles mixed with *E.coli* and *P. aureus* culture. Furthermore, the broth culture was incubated for 24 hours at 37˚C. Finally, the anti-microbial activity was checked by using UV-Visible spectrophotometer.

Anti-inflammatory activity:

Anti-inflammatory are compounds can be assessed using various methods, including Inhibition of protein denaturation assays. The anti-inflammatory activity of the bark sample of *Borassus flabellifer* was determined by using plant extract. In this assay bovine serum albumin (BSA) as standard protein. In test tubes add 900ml 5% BSA and 2.8ml PBS followed by each test tubes incubate at 37˚C for 15 minutes and heat 70˚C for 5 minutes after cool down the sample take reading at 660nm.

Formulation of Soap:

Soap is produced through the basic saponification reactions and the solidified basic soaps were immediately broken down to smaller pieces and melted in a water bath. The 5mg of the silver nanoparticles of *Borassus flabellifer* bark powder was calculated based on the herbal content of commercial herbal bath soap.

Physical & Chemical Examination:

Physical and chemical was done to evaluate the soap's pH, physical appearance, high temperature, fatty matter, moisture content, basicity, hardness foam retention, and basicity.

Biological Examination:

Thumb impression test and antimicrobial activity was done to check the efficacy of soap.

3. RESULT AND DISCUSSION:

Herbal soap was prepared using the silver nanoparticles synthesized from aqueous bark extract of *B. flabellifer* and characterization of silver nanoparticles using *B. flabellifer* (bark) are discussed in detail in the following chapter.

Phytochemical studies of *Borassus flabellifer:*

The phytochemical analysis of aqueous extract of bark of *B. flabellifer* was performed and reported in Table 1.

S. no	Phytochemicals	Result
1.	Alkaloids	+
2.	Flavanoids	+
3.	Steroids	+
4.	Terpenoids	+
5.	Saponins	-
6.	Tanins	+
7.	Phenol	+
8.	Resins	+
9.	Proteins	+
10.	Carbohydrates	+

Table 1: Phytochemical analysis of aqueous bark extract *of B. flabellifer* where,
(+) indicates the presents and (-) indicates absence of phytochemicals

Synthesis of silver nanoparticles from *Borassus flabellifer:*

Silver nanoparticles were synthesis and results were noted. Light brown to dark brown colour change indicates the formation of nanoparticles. Followed by centrifuged and pellets were dried and collected as powder.

Characterization of silver nanoparticles:

1. UV-Visible spectrophotometer:

The green synthesis of silver nanoparticles (AgNPs) using aqueous bark extract of *B. flabellifer* was studied. Formation of silver nanoparticles were confirmed by UV-Visible spectrophotometer (Figure. 1).

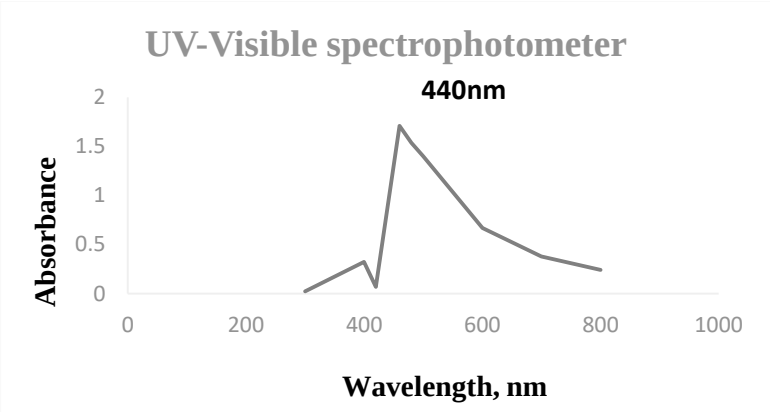

Figure 1: UV-Visible spectrophotometer of AgNPs synthesized using *B. flabellifer*

2. FTIR analysis of AgNPs of aqueous bark extract of *B. flabellifer*:

FTIR analysis was carried out to know the possible interaction between functional groups aqueous bark extract of *B. flabellifer* as nanoparticles (Figure 2). The spectral data were in the wavelength range of 3800-400 cm^{-1}. The FTIR spectrum of bark showed peaks around at 3919.35, 2330.01, 686.66, 648.08, 555.50 and 493.78 cm^{-1} (Table 2 and Figure 2).

Table 2: Functional group analysis of Silver NPs of *Borassus flabellifer* from FTIR spectroscopy

S.no.	Frequency Cm^{-1}	Bond	Functional group
1.	3919.35	O-H Stretching	Alcohol
2.	2330.01	C≡C Stretching	Alkynes
3.	686.66	C-H bonds	Benzene
4.	648.08	C-H bonds	Aliphatic alkane
5.	555.50	O-H Stretching	Hydroxyl group
6.	493.78	C-I Stretching	Halo compound

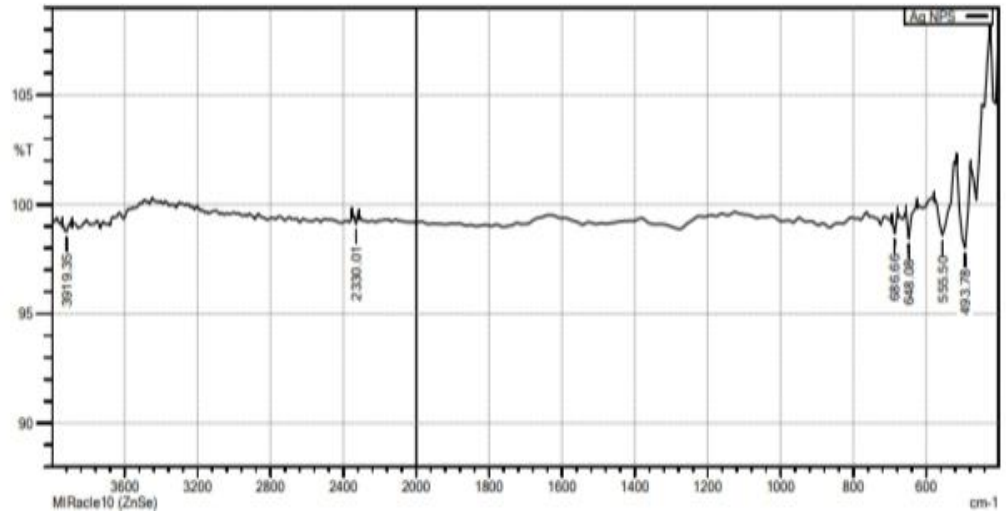

Figure 2: FTIR results of AgNPs of *Borassus flabellifer*

3. FE-SEM analysis of AgNPs of aqueous bark extract of *B. flabellifer*:

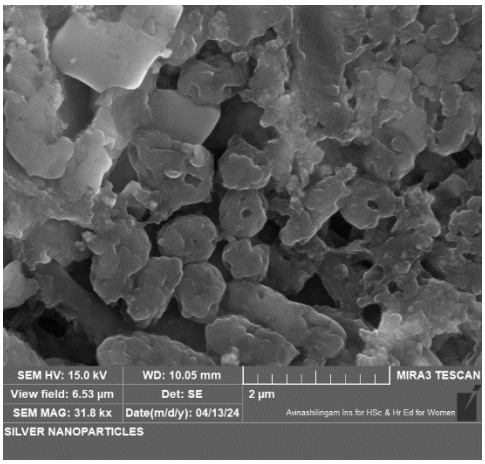

Figure 3: FE-SEM results of AgNPs of *Borassus flabellifer*

FESEM technique was used to visualize the morphology of AgNPs. Fig 6 shows the size and structure of the particles, as per the analysis, was highly crystalline and spherical in shape and are in the range of 200 nm in size indicating polydispersity. A few aggregations of SNPs are also observed in some places.

Biological assay:

Antimicrobial activity: The antimicrobial activity of silver nanoparticles were checked and the results were noted. The study was done by Turbidity assay method.

Furthermore, the broth culture was incubated for 24 hours at 37℃. Each conical flask shows the increase in turbidity of *E. coli* and *Staphylococcus aureus* cultures. Finally, the anti-microbial activity was checked by using UV-Visible spectrophotometer reading at 600nm.

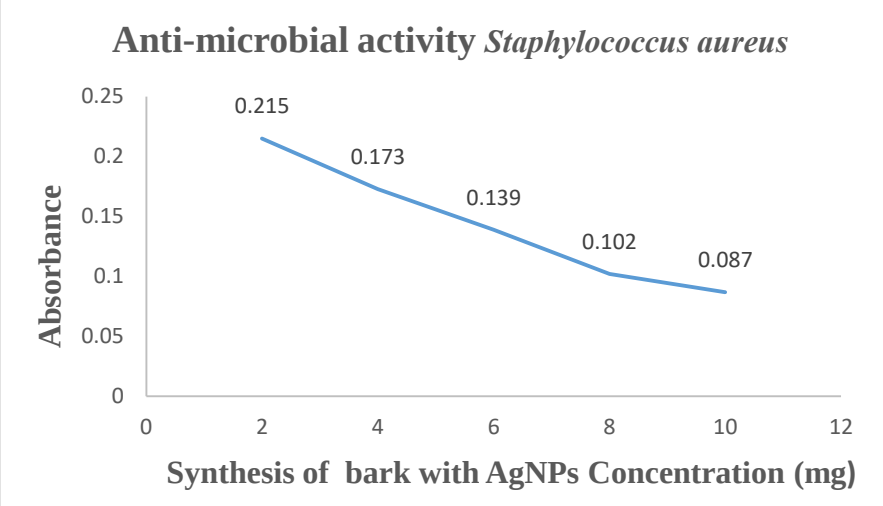

Figure 4: Antimicrobial activity of AgNPs synthesized from bark of *B. flabellifer* ***(S. aureus)***

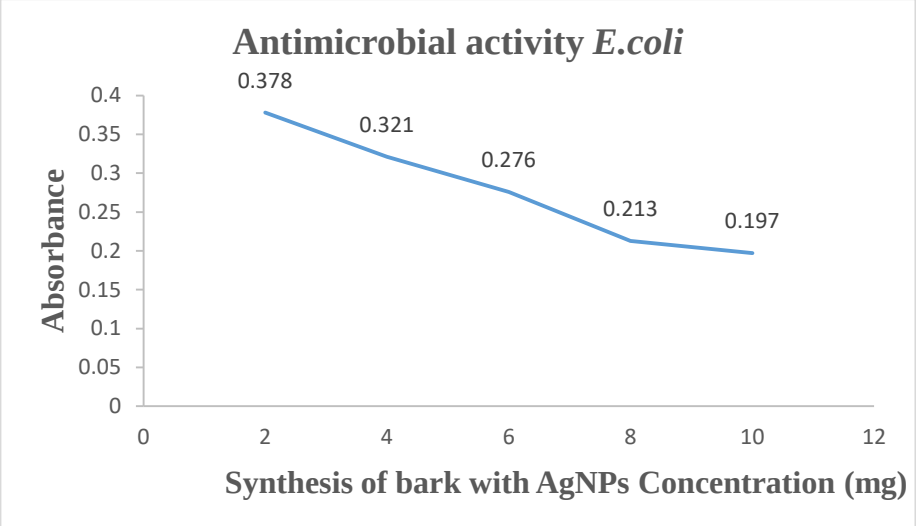

Figure 5: Antimicrobial activity of AgNPs synthesized from bark of *B. flabellifer*

Turbidity assay for antimicrobial activity shows a decrease in value, it typically indicates that the antimicrobial agent is effective in inhibiting microbial growth.

Anti-inflammatory assay:

To determine the anti-inflammatory property, the protein denaturation assay was determined by using in AgNPs synthesized from bark of the candidate plant which showed remarkable results as described below.

Extract of bark	**Concentration (mg)**	**Absorbance at 660nm**	**% of inhibition**
Control	**-**	**0.00**	**-**
Borassus flabellifer	**400**	**0.590**	**78.3%**
	600	**0.483**	**82.3%**
	800	**0.340**	**87.5%**
	1000	**0.109**	**96%**
Diclofenac sodium	**1000**	**0.200**	**95%**

Table 3: Protein denaturation assay

The increase in the concentration of sample i.e., indicates the protein denaturation, 62.3%, 78.3%, 82.3%, 87.5%, and 96% which shows the sample has great anti-inflammatory potential.

Evaluation of herbal soap:

After 24 hrs the soap solidifies it undergoes for physical, chemical and biological examination, which results the herbal soap is compared with neutral soaps shows the herbal shows good results.

Physical examination:

Characteristics	**Herbal soap**	**Neutral soap**
Odour	Good	Average
Shape	Oval	Oval
Hardness	Hard	Smooth
pH	7.4	9. 76
Foaming ability	Good	Average
Washing ability	Good	Average
% Foam retention	76%	56%
High temperature retention	54℃	44℃
% Moisture content	13.04%	12.44%

Table 4: Physical characteristics of the prepared herbal soap, compared with neutral soap

Chemical Examination:

Sample	% Fatty matter	Basicity	Grades
Herbal soap	76.00	No color change	I
Neutral soap	67.76	No color change	II

Table 5: Chemical characteristics of the prepared herbal soap, compared with Neutral soap

Biological Examination:

The formulated herbal soap shows good antibacterial activity against microorganisms is compared with neutral soap.

Disc diffusion method: The soap extract produced zone of clearance against Gram negative organism such as *E. coli* and antibiotic chloramphenicol (30mg) disc is used for the comparison, methanol used as negative control, Gram positive organism is *Staphylococcus aureus* which is compared with and Gentamycin (30 mg) antibiotic discs, methanol is used as negative control which is resistant against them.

	Zone of inhibition (diameter in mm)		
Micro-Organisms	**Antibiotic disc**	**Herbal soap**	**Neutral soap**
Staphylococcus aureus	31mm	33mm	31mm
Escherichia coli	23mm	29mm	28mm

Table 6: Antibacterial activity of formulated herbal soap comparing with neutral soap

Thumb impression test:



Figure 6: Thumb impression test

The **Fig 6** shows that the herbal soap is having good antimicrobial activity compare the neutral soap. (D) as Control, and Unwashed hands having the microbe's formation and shows the washed hands with neutral soap (B) shows less activity but that (C) herbal soap shows good removal of microbes and dirt from hands.

5. SUMMARY AND CONCLUSION:

Synthesis of Silver nanoparticles from the aqueous extract of bark of *Borassus it flabellifer* led to the formulation of herbal soap. After UV Visible spectrophotometry, FTIR, FE-SEM, Anti-inflammatory, Anti-microbial assay and evaluation of soap it proved to be a better candidate for formulation of soap compared to neutral soap. Plant bark shows highly effective antimicrobial soaps perform recovery from allergy, protect the skin, evading dirt and microorganism that can evaluated by physical, chemical and biological examination.

REFERENCE

[1] A Jerry, Comprehensive Review on the Medicinal Properties of *Borassus Flabellifer* PG & Research Department of Biochemistry, Sacred Heart College (Autonomous), Tirupattur-635601, TN, India, Received: October 15 2018/Accepted: 27 November 2018/Published: 07 December (2018).

[2] Saira Mariam Banu, Nora Vigasini, Shanmugapriya Surenderan, Phytochemical Screening, In Vitro Antioxidant And Anti-Inflammatory Activity Of Freeze-Dried *Borassus Flabellifer L.* Seed Powder, Asian Journal Of Biological And Life Sciences 10 (1), 203, (2021).

[3] N Thirumagal1, and A Pricilla Jeyakumari1. Photocatalytic and Antibacterial Activity of Silver Nanoparticles (Agnps) Using Palm (*Borassus Flabellifer L.)* Water Pathaneer Department of Physics, Thiruvalluvar Government Arts College, Rasipuram, Tamil Nadu, India Thirumagal and Jeyakumari / Indian Journal of Science and Technology; 13(18):1856–1866, (2020).

[4] Handa SS, Khanuja SPS, Longo G, Rakesh Dd. Extraction Technologies for Medicinal and Aromatic Plants. Inter Cent Sci High Tech, 5(6): 21-25, (2005).

[5] Prasad G Jamkhande, Vikas A Suryawanshi, Tukaram M Kaylankar, and Shailesh L Patwekar. Biological Activities of Leaves of Ethnomedicinal Plant, *Borassus Flabellifer Linn*.(Palmyra Palm): An Antibacterial, Antifungal And Antioxidant Evaluation, Bulletin Of Faculty Of Pharmacy, Cairo University 54 (1), 59-66, (2016).

[6] J Sandhya and S Kalaiselvam. Biogenic Synthesis of Magnetic Iron Oxide Nanoparticles Using Inedible *Borassus Flabellifer* Seed Coat: Characterization, Antimicrobial, Antioxidant Activity And In Vitro Cytotoxicity Analysis, Res. Express 7(1), 015045, (2020).

[7] Sangita Chandra, Priyanka Chatterjee, Protapaditya Dey, and Sanjib Bhattacharya. Evaluation of *In Vitro* Anti-Inflammatory Activity of Coffee Against The Denaturation of Protein, Pharmacognosy Division, Bengal School of Technology (A College of Pharmacy), Delhi Road, Sugandha, Hooghly 712102, West Bengal, India, Asian Pacific Journal of Tropical Biomedicine S178-S180, (2012).

[8] Rios J L and Recio M C, Medicinal Plants and Antimicrobial Activity. J Filmopharmacol. 100(1-2): 80-84, (2005).

[9] Kantrao Saware, Balaji Sawle, Basavraja Salimath, Kamala Jayanthi and Venkataraman Abbaraju. Biosynthesis and Characterization of Silver Nanoparrticles using *Ficus benghalensis* Leaf extract. International Journal of Research in Engineering and Technology. 3(5), 2321-7308, (2014).

[10] Ashok K R & Ramaswamy M, Phytochemical Screening by Ftir Spectroscopic Analysis Of Leaf Extracts Of Selected Indian Medicinal Plants. Inter J Cur Micro App Scí, 3(1): 395-406. (2014).

[11] Latif Ahmed, Mu Hazarika and Dhrubajyoti Sarma. Formulation And Evaluation of An Ayurvedic Bath Soap Containing Extracts of Three Ayurvedic Herbs, Journal of Medicinal Plants Studies; 9(2): 115-117, (2021).

[12] Kandasamy R, Rengasamy K, Subramani S, Henry L, and Jeeva K J. Formulation Of Herbal Bath Soap From Vitex Negundo Leaf Extract, J Chem Pharma Sci. 11(5): 225- 232, (2014).