Journal of Engineering Sciences
Vol 11, Issue 4 ,April/2020
ISSN NO:0377-9254

"Green synthesis, characterization and biological activity of silver nanoparticles from Musa paradisiaca L. and Cynodon dactylon"

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***Abstract*— Silver nanoparticles were synthesized by using flower extracts of a *Musa paradisiaca* L. and *Cynodon dactylon* which reduces silver ions to silver nanoparticles. Synthesized nanoparticles were confirmed by colour change of plant extracts. The prepared silver nanoparticles showed an absorption band at 491nm and 370.50 nm and 536nm and 397nm of methanolic and aqueous extracts of *M. paradisiaca* and *C. dactylon* respectively. Four different concentrations of nanoparticles of MeOH and Aq extracts of both plants have been evaluated for their *In vitro* anti-inflammatory activity. All test samples except 1000 µg/ml of Aq extract of M. *paradisiaca* exhibited significant membrane stabilization activity, when compared with control. In all test concentrations high concentration (10000 µg/ml) of MeOH extract of *M. paradisiaca* was more effective (93.91 ± 0.167) when compared to control. *M. paradisiaca* exhibited more potent anti-inflammatory activity as compared to *C. dactylon*. Thus, the present study concludes the Aq and MeOH extracts of *M. paradisiaca* and *C. dactylon* having potential anti-inflammatory effect.**

***Keywords*— Silver nanoparticles, *Musa paradisiacal* L., *Cynodon dactylon*, Anti-inflammatory, FT-IR**

1. INTRODUCTION

In the past, silver was used for a variety of clinical conditions including epilepsy, venereal infections, acnes and leg ulcers. In the recent years nanoparticles synthesis using plant sources are gaining more interest, specifically the use of various parts of the plants such as leaf (Shiv Shankar *et al*., 2004; Krishnaraj *et al*., 2010; Begum *et al*., 2009; Song and Kim, 2009; Huang *et al*., 2007; Raut *et al*., 2009; Garg, 2013), corn (Garg,2012), tuber (Sathishkumar *et al*., 2010), bark (Sathishkumar *et al*., 2009) and buds (Raghunandan *et al*., 2010). Reported studies related to biological syntheses of SNPs especially using medicinal plants have been promising (Krishnaraj *et al*., 2010; Song and Kim, 2009; Sathishkumar *et al*., 2009; Satyavani *et al*., 2011; Jha *et al*., 2009; Thakkar *et al*., 2010). The methods using plant extracts involve phytochemicals such as terpenoids (Thakkar *et al*., 2010), flavonoids (Raghunandan *et al*., 2010), phenol derivatives (Jacob *et al*., 2011), plant enzymes (hydrogenases, reductases, quinones) and their derivatives, di-hydric phenols (Jha *et al*., 2009) and so on act as reductants in the presence of metal salt.

These medicines are safe in therapeutics. Moreover, nano silver exhibits remarkable biological properties, such as antiviral activities (Foldbjerg *et al*., 2009 and Duran *et al*., 2007), anthelmintic activity (Garg and Chandra 2012) and analgesic activity (Garg *et al*., 2014).

Banana (*Musa paradisiaca*) is a familiar tropical and largest herbaceous flowering plant. The fruit of *M. paradisiaca* and *M. sapientum* is traditionally used in diarrhoea (unripe), dysentery, intestinal lesions in ulcerative colitis, diabetes (unripe), in sprue, uremia, nephritis, gout, hypertension, cardiac disease. Banana leaves (ashes) are used in eczema, as cool dressings for blister and burns. Flowers are used in dysentery and menorrhagia. Pseudo stem juice of fruited plant is used for treating diarrhea, dysentery, cholera, otalgia, haemoptysis. The root is used as anthelmintic, blood disorders, venereal diseases. The plant is also used in inflammation, pain and snakebite (Divya *et al*., 2016).

Cynodon dactylon belongs to family of Poaceae and is termed as a creeper in India (Asthana *et al*., 2012). The plant is traditionally used as an agent to control diabetes in India. The extract of *C. dactylon* leaf has been reported to be antidiabetic,

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antioxidant and hypolipidemic efficacy, healing of minor injuries, immunomodulatory and hepatic antioxidant activities. The aqueous fluid extract of *C. dactylon* rhizome is used for diuretic, anti-emetic, purifying agent and dysentery. The plant extract also has significant application in dropsy and secondary syphilis, wounds, and cardio protective. In a recent study, the extracts of *C. dactylon* had also been reported to be effective for antimicrobial activity against bacterial pathogens and fungus (Kaliyaperumal *et al*., 2013).

At present, due to modern food practices the interest to consume banana flowers is found to be declining and based on the aforementioned comments, it is not surprising that the pharmacological benefits of *C. dactylon* have been attracting great interest. Since inflammation are the root cause for many human ailments, the proposed study is designed in such a way to provide scientific evidence to prove the anti-inflammatory potentials of *Musa paradisiaca* and *C. dactylon in vitro* by synthesizing Silver Nanoparticles.

2. Materials and Methods

Collection of plant

The plants were collected from North Maharashtra Region in the period of September 2017. The plants were identified by Dr. Tanveer Khan, Department of Botany, H. J. Thim College, Jalgaon and deposited a voucher specimen in the Department of Zoology.

Preparation of Extract

The plants materials were collected from North Maharashtra Region Jalgaon District, Maharashtra State, India. The flowers of *Musa paradisiaca* and whole plant of *C. dactylon* were shade dried. After complete drying the material was crushed and grinded to form coarse powder. One kg of dried powdered plant material was exhaustively extracted in Soxhlet apparatus with methanol and distilled water. The solvent extract so obtained was then filtered to remove any suspended impurities. Extract was concentrated under reduced pressure and controlled temperature (55^0C to 60^0C). The extract of plant was preserved in dry, cool condition in desiccator. Thus the methanolic (MeOH) and aqueous (Aq) extracts obtained were used to biosynthesis of silver nanoparticles and further proceed for their *in-vitro* anti-inflammatory activity.

Synthesis of AgNPs and Characterization of AgNPs

Silver nitrate (AgNO3) of analytical reagent grade was used as a precursor for the synthesis of AgNPs. Silver nanoparticles were prepared by adding 5 mL of the extract (of respective concentration) to 1 mL of aqueous silver nitrate solution (1M) at room temperature. The mixture was kept for 10 minutes on rotary shaker and kept in the laboratory at room temperature. The color change was continuously observed.

Characterization of AgNPs

Silver nanoparticle synthesized was confirmed and characterized by following methods; colour change, UV-Vis Spectrophotometer and Fourier transformed infrared (FTIR) spectrum. The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium after 24 h after diluting 0.5ml of the sample with 1.5 ml sterile distilled water. UV-Vis spectral analysis was done by using UV-Vis Spectrophotometer. Fourier transformed infrared (FTIR) spectrum of the sample was recorded.

Protocol for Anti-inflammatory activity

The anti-inflammatory activity was evaluated using RBC membrane stabilization method (Divya *et al*., 2016). Blood sample (2 ml) was collected from volunteer in a heparinized tube and washed with PBS twice and centrifuged at 3000 rpm for 10 min (Centrifuge Make: Remi, Model R303). Then RBC was suspended in phosphate buffer, pH 7.2 and taken in a tube (0.5 ml) with 0.5 ml of extract and 0.5 ml hypotonic solution and incubated for 30 min at room temperature. Then the contents were centrifuged at 1500 rpm for 10 min and the supernatant was collected and the absorbance was read at 560 nm. Based on the absorbance of extract and control, the membrane stabilization effect was calculated and expressed on percentage basis.

Statistical analysis

All data were expressed as mean ± SE and the ANOVA were applied to determine the significance of the difference between the control group and experimental groups.

3. Results

Colour change

As the extract was added to aqueous silver nitrate solution, the transparent light brown color solution was changed into the dark brown color. This change in color is an indication of the formation of AgNPs. Due to splitting of $AgNO_3$ into Ag+ and NO_3^- change in colour of the reaction mixture was

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observed, with progressive time. Apparently, the metabolites in the extract acted as e- donor and reduce Ag+ ions into Ag. Consequently, the formation of nanoparticles was indicated by brown colour of the aqueous solution following the excitation of surface Plasmon vibrations. Change in colour of the reaction mixture during the time of incubation indicated the formation of silver nanoparticles and was confirmed by the characteristic peaks obtained by UV–visible spectra analysis. It can be predicted that the formation of nanoparticles occurs due to the presence of alkaloids, phenols, nitro groups and amines, which are abundantly found in MeOH and Aq extracts of *M. paradisiaca* and *C. dactylon* mediated silver nanoparticles and are also confirmed from the FTIR spectra.

FT-IR spectrum

FTIR is an important tool which enables us to understand the involvement of functional groups in the interactions between metal particles and biomolecules. In the present work, FTIR spectra are used in the identification of biomolecules responsible for capping and stabilizing the silver nanoparticles. The resulting nanoparticles were characterized through FT-IR analysis. Based on absorbance bands different resultant groups were identified, which was assigned to the stretching vibrations of primary and secondary amines, respectively. The FTIR spectra of reduced silver nitrate show considerable variation in the peaks of spectra (Fig 1, 2). The nanoparticles of MeOH and Aq extracts display a number of absorption peaks, reflecting its complex nature. The reduction of certain peaks is the clear indication of the loss of certain groups.

UV-vis spectral study

The prepared silver nanoparticles mediated by MeOH and Aq extracts *M. paradisiaca* showed an absorption band at 491 and 370.50 nm as shown in Fig 3. Similarly, the prepared silver nanoparticles mediated by MeOH and Aq extracts of *C. dactylon* showed an absorption band at 536nm and 397nm of methanolic and aqueous extract respectively as shown in Fig 4, which is a typical absorption band of spherical Ag nanoparticles due to their surface Plasmon. The absorption band in visible light region (350 nm – 550 nm) is typical for silver nanoparticles (Lee and Meisel, 1982).

Anti-inflammatory activity

Four different concentrations of nanoparticles of MeOH and Aq extract of *M. paradisiaca* and *C. dactylon* have been evaluated for their *In vitro* anti-inflammatory activity.

In all test concentrations of *M. paradisiaca*, high concentration of MeOH extract (10000 µg/ml) was more effective (93.91 ± 0.167) when compared to control. The 2500 and 5000 µg/ml of MeOH and Aq extracts exhibited very close % of inhibition membrane stabilization activity. All test samples except MeOH 1000 µg/ml exhibited significant (***P<0.05) membrane stabilization activity. All test samples exhibited significant membrane stabilization activity, when compared with control.

The results of *C. dactylon* exhibits significant membrane stabilization activity, when compared with control. High concentration of Aq extract mediated silver nanoparticles at 10000 µg/ml concentration was more effective (67.74 ± 0.123) as compared to all remaining concentrations of both extracts. The MeOH extracts mediated AgNP exhibited membrane stabilization activity as dose dependent. At the dose 2500 and 5000 µg/ml of Aq extract mediated AgNP revealed a very close % of inhibition. At 10000 µg/ml of nanoparticles of MeOH and Aq exhibited % inhibition 62.85 and 67.74 respectively (Table 1); are significantly different than standard.

However, it is interesting to note that, the standard is a pure compound, whereas, the methanol and aqueous extracts contains complex of active ingredients. Therefore, it is noteworthy that, purification of active principle from extract will probably enhance the membrane stabilization activity than standard.

4. Discussion

The synthesis of nanoparticles is attracting attention because of their usually enhanced physiological properties and biological activities as compared to the bulk parent material (Tolaymat *et al.*, 2010). Biological synthesis is preferred for being cost effective and the ability to maintain the homogeneity and stability of the synthesized nanoparticles. The unarguable property of silver nanoparticles of being strongly antibacterial makes it more favorable for the use in medical devices and supplies such as wound dressings, scaffolds, skin donation, recipient sites, and sterilized materials in hospitals, medical catheters, contraceptive devices, surgical instruments, bone prostheses, artificial teeth and bone coating. Apart from this the naoparticles of

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such herbal extracts act as an analgesic, anti-inflammatory and antipyretic too. This is supported by the study carried out by Garg *et al*., (2014). They studied analgesic potential of hydrogels of silver nanoparticles using aqueous extract of *Saraca indica* bark. Our finding is corroborated with findings of Garg *et al*., (2014) though they evaluate analgesic activity of nanoparticles of different plants, because analgesic, anti-inflammatory and antipyretic activities are correlated with each other.

5. Conclusion

The rate of reaction for the biosynthesis of these nanoparticles is rapid and ecofriendly. Which can be used as an alternative to chemical synthesis protocols at low cost. Nanoparticles synthesized from MeOH and Aq extracts of *M. paradisiaca have* significant *in vitro a*nti-inflammatory activity. Nanoparticles of *M. paradisiaca* may have persuasive application in medicine therapeutics and diagnostics.

There is a growing need to develop clean and nontoxic procedures for synthesis and assembly of nanoparticles and the MeOH and Aq extracts obtained from *C. dactylon* are capable of producing silver nanoparticles extracellular and are quite stable in solution. The rate of reaction for the biosynthesis of these nanoparticles is rapid and ecofriendly, which can be used as an alternative to chemical synthesis protocols at low cost. Metal nanoparticles by herbal approach reported in the present study using *C. dactylon* may have persuasive application in medicine therapeutics and diagnostics.

6. ACKNOWLEDGMENTS

The authors are thankful to the Principal, Moolji Jaitha (Autonomous) College, Jalgaon, Maharashtra, India for providing necessary facilities. Authors are also thankful to Dr. Ganesh Chaudhari, for their kind cooperation for FTIR and UV spectrum analysis.

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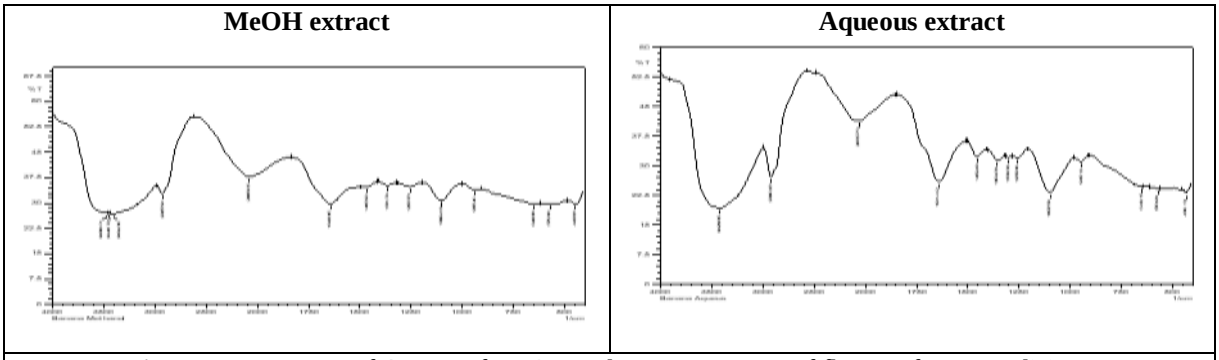


Fig. 1 FT-IR spectra of AgNPs of MeOH and aqueous extract of flower of *M. paradisiaca*

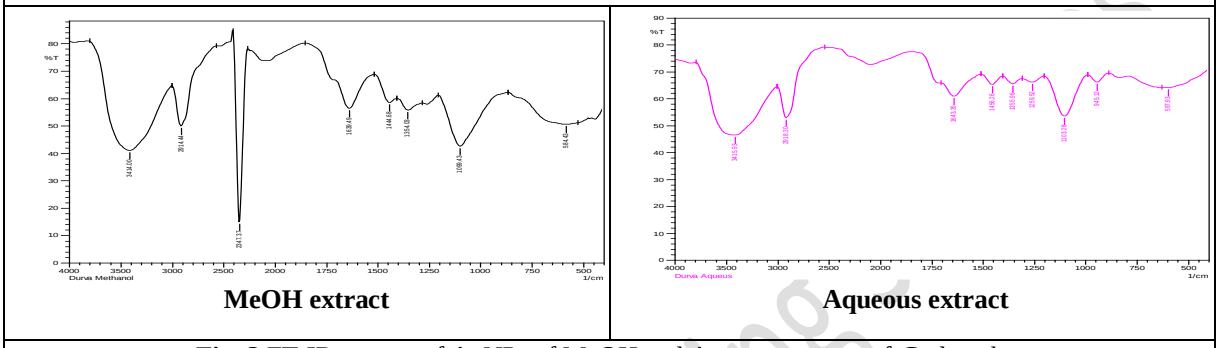
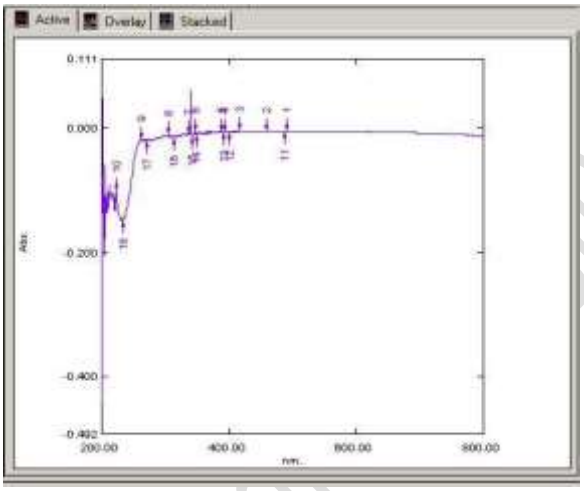


Fig. 2 FT-IR spectra of AgNPs of MeOH and Aqueous extract of *C. dactylon*

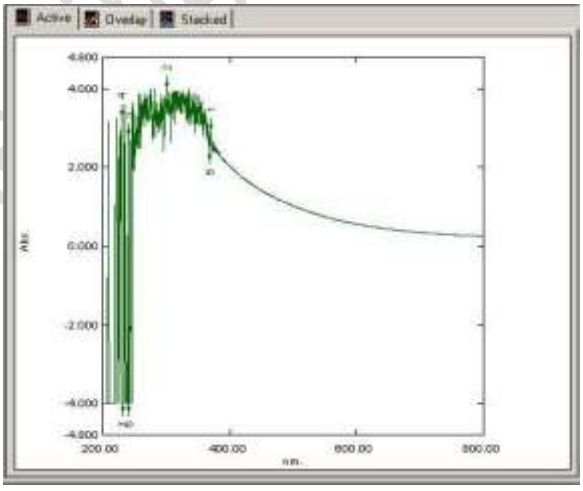
Table 2 FT-IR spectrum analysis of synthesized silver nanoparticles from extracts of *M. paradisiaca* and *C. dactylon*

Sr. No.	Wave Number	Stretching	Interpretation
Silver nanoparticles mediated from aqueous extract of M. *paradisiaca*			
1.	3423.65	Varies o-H Stretch	Alcohols
2.	2920.23	CH stretch	Alkanes
3.	2005.76	N=C in R-N=C=S	Misc
4.	1841.42	C=O stretch	Acid chloride
5.	1452.40	CH2 and CH3	Alkanes
6.	1354.03	Arom Nitro	Misc.
7.	1360.02	Ar-N stretch	Amines
8.	1255.66	-CH3	Alkanes
9.	1097.50	C-O stretch	Alcohols
10.	945.12	N-O aliphatic	Misc.
Silver nanoparticles mediated from methanolic extract of M. *paradisiaca*			
1.	3468.01	O-H Stretch	Alcohols
2.	3448.72	N-H stretch	Amines
3.	3404.36	Monomer OH, NH Stretch	Carboxylic acids, Amides
4.	2927.94	-CH2-	Alkanes
5.	2085.05	N=C in R-N=C=S	Misc.
6.	1643.35	C=C or C=O stretch	Alkenes, Amides
7.	1465.90	-CH2-	Alkanes
8.	1361.71	S=O sulfonyl chloride 1	Misc.
9.	1253.73	Si-CH3 (sharp)	Misc.
10.	1099.43	C-O stretch	Alcohols

Silver nanoparticles mediated from aqueous extract of *C. dactylon*			
1.	3455	O – H stretching	Alcohol
2.	2913	C – H stretching	Alkane
3.	1643	C = C stretching	Alkene
4.	1456	C – H bending	Alkane
5.	1366	C – H bending	Alkane
6.	1258	C – H bending	Alkane
7.	1103	C – O stretching	Alcohol
8.	945	C = C bending	Alkene
Silver nanoparticles mediated from methanolic extract of *C. dactylon*			
1.	3414	N – H stretching	Primary amine
2.	2914	N – H stretching	Amine salt
3.	2347	C=O=O	CO_2
4.	1639	C = C stretching	Alkene
5.	1444	C – H bending	Alkane
6.	1354	S = O stretching	Sulfonate
7.	1099	C – O stretching	Ether

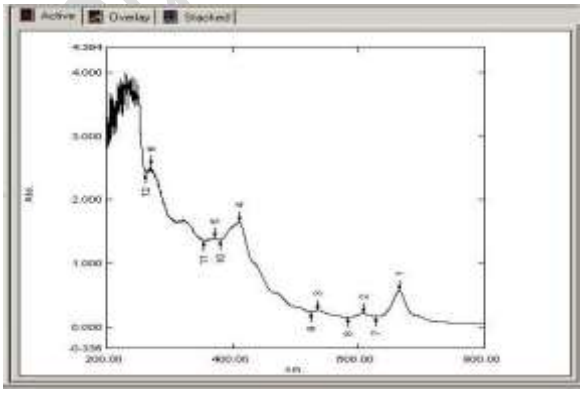


491.00 nm for Methanol extract

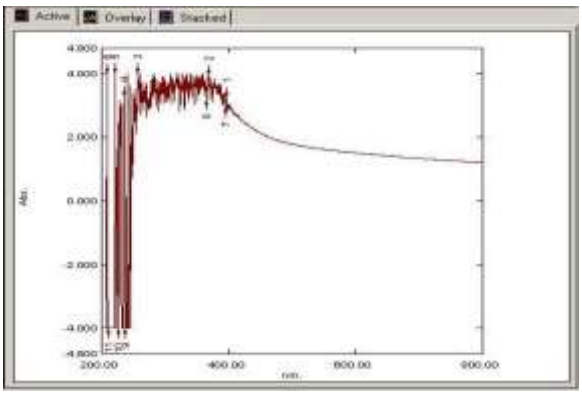


370.50 nm for Aqueous extract

Fig. 3 UV-Vis spectra of the AgNPs using MeOH and Aq of *M. paradisiaca*

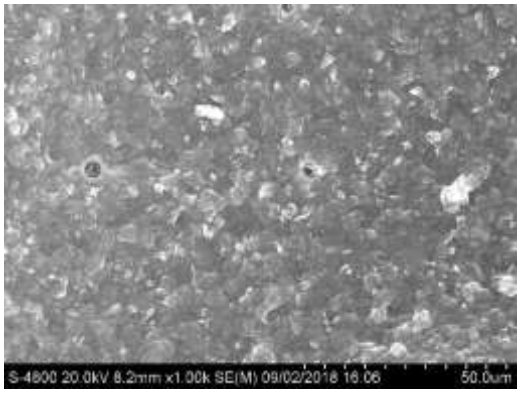


536.00 nm for Methanol extract

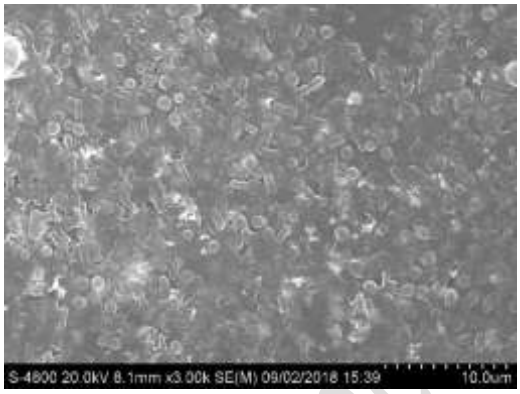


397.00 nm for Aqueous extract

Fig. 4 UV-Vis spectra of the silver nanoparticles of MeOH and Aq extracts of *C. dactylon*

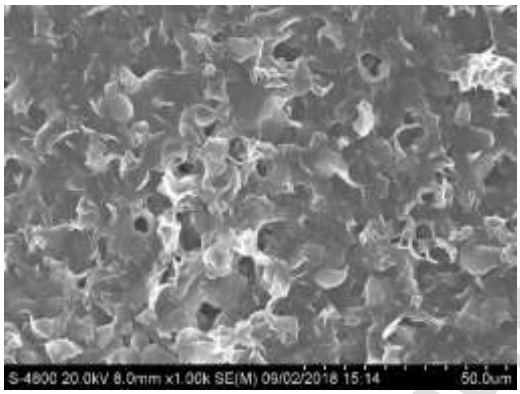


Methanol extract

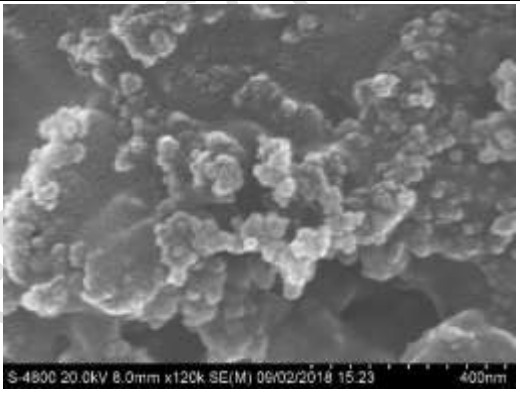


Aqueous extract

Fig. 5 TEM of the formed AgNPs using MeOH and Aq of *M. paradisiaca*



Methanol extract



Aqueous extract

Fig. 6 TEM image of AgNPs from MeOH and Aqueous extract of *C. dactylon*

Table 3 *In Vitro* Anti-inflammatory activity of the nanoparticles mediated by *M. paradisiaca* and *C. dactylon*

Groups	Conc. 'µg/ml'	% Inhibition	
		M. paradisiaca	*C. dactylon*
Control	------	00.00 ± 0.00	00.00 ± 0.00
Standard	50	98.66 ± 0.088	98.66 ± 0.088
MeOH	1000	07.84 ± 0.214***	13.19 ± 0.108*
	2500	22.79 ± 0.246***	29.1 ± 5.495***
	5000	29.91± 0.287***	58.15 ± 4.808***
	10000	93.91 ± 0.167***	62.85 ± 0.096***
Aq	1000	01.86 ± 0.216	9.21 ± 0.065***
	2500	25.94 ± 0.283***	23.04 ± 0.371***
	5000	30.85 ± 2.566***	23.25 ± 0.100***
	10000	43.13 ± 0.218***	67.74 ± 0.123***

Each value expressed as mean ± SE, n=6, ***P<0.001 and *P<0.05 Vs Control.

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