Nutraceutical Value of *Ganoderma* species from Melghat Forest of Maharashtra

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Abstract : Ganoderma are poroid fungi which shows higher medicinal properties. Melghat located at central India present at satpura range in Maharashtra. Ganoderma are frequently grown at Melghat region. Melghat region having victims of Malnutrition. Ganoderma may be helps to reduce malnutrition. Study of Three species in Ganoderma that is Ganoderma lucidum, Ganoderma applantum and Ganoderma multiplicatum shows Nutritional, Medicinal, Antibacterial, Antioxidants values. Ganoderma lucidium shows higher number of nutritional value such as Carbohydrates, Proteins, Fiber, Vitamin C, Polysaccharides, etc as compare to Ganoderma applantum and Ganoderma multiplicatum. Secondary metabolites such as Alkaloids, Flavonoids, Triterpenoids and so many metabolites are present in Ganoderma. Ganoderma lucidum shows higher amount of Scavenging activity against DPPH free radical. All three species of Ganoderma shows tremendous Antibacterial activity against *E.coli. Pseudomonas aeruginosa, Klebsiella pneumonia* and Staphylococcus aureus. Such type of nutritional as well as nutraceutical value containing food may be use to reduce malnutrition in Melghat.

IndexTerms : Ganoderma , Nutritional value , Antioxidant , Antibacterial , FTIR , Malnutrition.

I. INTRODUCTION

Ganoderma is polyporous mushroom belonging to family Ganodermataceae. *Ganoderma* is wood rotting fungi because of its saprophytic as well as parasitic nature. *Ganoderma* is generally growing on living as well as dead wood. It is generally grown at the base of stump. *Ganoderma* is first nominated by Petter Adolf Karsten in 1881.William Curtis gave first binomial name Boletus lucidusin1781.Due to shiny or leathery surface give name *Ganoderma lucidum*. *Ganoderma lucidium* is classified under division of Basidiomycota. The woody bracket is called corks. It's stem called as stype. Double wall spores shows member of family Ganodermataceae.

Melghat is the located at North region of Amravati in Maharashtra. It is sited in Satpura Range. Melghat is very diversified region for Mushrooms. There are 780 types of Mushrooms already studied in Melghat region. Melghat having favorable condition in August to January for Mushrooms. Number of species in genus *Ganoderma* are identified in Melghat. But this beautiful natural place having evil eye of malnutrition. In that condition they required highly nutritional as well as easily available food.

Ganoderma lucidum contain more number of nutritional value which is helpful for victim of malnutrate people. As comes as malnutrition people also having other issues such as lower immunity, illness, bleeding, etc. So that condition medicinal properties containing food which shows both Nutritional as well as medicinal properties like *Ganoderma lucidum*. *G. lucidum* having numerous medicinal activity. It contains metabolite such as Glycosides, Terpenoids, Phenolic compounds. Some essential metabolite which is use to cure infection. *Ganoderma lucidum* shows sensitive activity against pathogenic organism such as gram positive and gram negative bacterias like *E. coli*, *S. aureus, Pseudomonas aeruginosa*, etc. Most of problem is occur due to free radicals such as weakness in eye, heart, skin, etc. So that issue solve by antioxidants. Antioxidant is biological components which tackle free radicals attacking on body. This is analyze by DPPH method DPPH is 2,2 diphehyl 1 pycrylhydrazyl is the free radical is use to calculate amount of free radicals tackle by antioxidants present in *G. lucidum*.

II. MATERIAL AND METHOD

Study Area : Melghat is located at 21°26′45″N 77°11′50″E in northern part of Amravati District of Maharashtra State in India. Melghat is one of the most favorable place for the host of *Ganoderma*. Verious poroid members are collected from the Melghat region. Melghat shows diversity of fungi due to the environmental conditions such as temperature, moisture, humidity, etc. It is most suitable environmental condition for porous fungi. Another member of poroid fungi from members of Family *Ganodermataceae, Hymenochaetaceae* are also commonly occurred in Melghat.

Collected specimens were checked by hand lens and select basidiocarps and packed in brown paper envelops and assigned a temporary number. Information regarding type of host, type of forest, name of the locality, date of collection was noted in field note-book. After removing extraneous matter, the basidiocarps were examined with hand lens and the details regarding the size and type of basidiocarp, colour of the hymenial and abhymenial surfaces, shape and number of pores per mm, tube length and type of margins noted down and taken photograph and measurements immediately with the help of scale. Then in laboratory Condition first identified sample.

Drying and Preservation: For further analysis collected basidiocarps were dried either in the sun or shed dry on blooming paper or by using portable electric drier. The dried specimens were packed in zipper packets of required size. To protect the specimens from attack of insects and worms Dichlorobenzene crystals were added.

Method of extraction :

Powder were prepared with the help of grinder or Morter and Pester. After this further process it extracted by using Soxhelt Method. For the *Ganoderma lucidium* chosen Methanol solvent system and and for *Ganoderma applantum* and *Ganoderma multiplicatum* chosen Acetone, Ethyl Acetate, Ethanol and Water Solvent system . In the Soxhelt method after the preparation of extract it was evaporated by using rotating evaporator at 40°C Then it used in further biochemical process. (Franz von Soxhlet, 1879)

Extract was dried by using rotating evaporator at 50°C . Dried extract was used for further analysis and liquid extract is used for phytochemical Analysis.

Nutritional value

- 1. Total carbohydrates (Anthrone Method): 100 mg powder sample was hydrolysis in 5ml 2.5 N Hydrochloric acid holding in water bath for three hours neutralized with sodium carbonate. Made 100 ml volume and centrifuged it.0.5 and 1 ml aliquots were taken from supernatant for analysis. Prepare the sample by taking 0, 0.2, 0.4,0.6,0.8 and 1mlof working standard. Make up the volume up to 1 ml including samples then add 4ml of ice cooled reagent and heated in water bath for 8 minutes. Then cooled rapidly Green too dark green color was obtained then immediately taking the absorbance at 630nm.(Hedge and Hifreiter, 1962).
- 2. Proteins (Crude): 2 g of sample was taken in 500 ml Micro-Kjeldhl's flask then added 10 g of digestion mixture (9.5 g of Potassium sulphate and 0.5 g of Copper sulphate) and 20 mole of Concentrated Sulphuric acid. Small pieces of calcium carbonate present in the flask to check bumping. Then give heat by using heater or burner at low flame till the color became light bluish green. Then flask was cooled 8-9 hours diluted the material by distilled water to make the aliquot of 250 ml in volumetric flask.25 ml aliquot was taken in 300 ml of Micro Kjeldahl flask. 80ml of saturated sodium hydroxide solution was added and kept on heater with distillation unit and connected the condenser with tap. The Lower end of condenser was dipped in solution of 25 ml of 2% Boric acid containing Toshiro's indicator (Methyl red 80mg, Methylene blue 20 mg in 100 ml of Methanol) in 250 ml in beaker. Distillation was carried out for 30 minutes during all ammonia released was tapped in boric acid solution. Then calculate percentage of crude protein by multiplying total nitrogen by 4.38. Percentage of Crude Proteins = % of Nitrogen x 4.38 (for mushroom). (Anonymous; Association of official and Analytical Chemists, Official Methods of analysis of the Association of Official Analytical Chemists, 12 Edn, Washington D.C., USA, 1975).
- 3. Fiber : Extract 2g of dried material mixed with ether or petroleum ether to remove fat (Initial boiling temperature 35 -38°C and final temperature 52°C). if fat content is below 1%, extraction may be omitted. After extraction with ether boil 2g of dried material with 200mL of sulphuric acid for 30min with bumping chips. Filter through muslin and wash with boiling water until washing are no longer acidic. Boil with 200mL of sodium hydroxide solution for 30min.Filter through muslin cloth again and wash with 25mL of boiling 1.25% H2SO4, three 50mL portions of water and 25mL alcohol. Remove the residue and transfer to ashing dish (preweighed dish W1).Dry the residue for 2h at 130 ±2°C. Cool the dish in a desiccator and weigh (W2).Ignite for 30min at 600 ±15°C. Cool in a desiccator and reweigh (W3).

% crude fiber in ground sample =Loss in weight on ignition (W2 - W1) – (W3 - W1)x 100Weight of the sample (Maynard, A J (Ed) (1970) Methods in Food Analysis Academic Press New York).

- 4. Total Ash: 5 gm of dried sample was taken in weighed crucible. Then heated in muffle furnace (600+- 15°C) till content was free of black particles. Crucible was cooled and weighed to constant weight. The ash vale calculator by weighed crucible.
- 5. Simple carbohydrates: Analysis of simple carbohydrates was calculated by subtracting the value of Polysaccharides from Total amount of carbohydrates.
- 6. Reducing Sugar : Weighed 100 mg of dried sample and extraction of sugar with 80% ethanol. Collected Supernatant was evaporated by using water bath at 70-80° C addition of water to dissolve sugar. Prepared aliquot of 0.5-3.0 ml of extract in test tubes and make up the volume upto 3.0 ml with water. Then added 3ml of DNS reagent and give heat. Prepared 40% Rochelle salt solution and added 1ml in each test tube. Wait for cooling and Red Dark red color at 510 nm. Reading was taken against standard (Glucose).(Miller, 1972).
- 7. Total Polysaccharides : Extraction of all sugar Samples with help of 80% hot ethyl alcohol. Extraction of simple sugar was carried out until there was no coloration in elute by a throne reagent. Then All residue was hydrolysed by using hydrocloric acid (2.5 N) till all Polysaccharides converted into glucose and then glucose is treated with anthrone reagent and an concentrated acid. Green color was observed and take the reading by using spectrophotometer as Same as total carbohydrates (Hedge and Hofreiter, 1962).

Secondary Metabolite Profiling: Secondary metabolites play crucial role in plants as defense system as well as so many functions in plants. Like that secondary metabolites becomes used by animals in the form of medicine. Off this all Eight types of of secondary metabolites were studied. The standard methodology were followed (Sofowora1983; Kadabadi et al. 2011, Evans 2002, Ahmad 2007)

- 1. Saponin : 1ml of extract was added with 10 ml of distilled water, then continuous Shake up to 30-40 sec. Persistent frothing or foam has been form that's indicated the presence and amount of saponin in the sample.
- 2. Alkaloid: 2 ml of extract was taken in the test tube, then hydrolyzed by 1% Hydrochloric acid, then added Mayer's, Dreagendorff's or Wagner's reagent in the sample then creamish, brown red or orange precipitate indicates the presence and amount of alkaloids present in the sample.
- 3. Flavonoids : 0.2 ml of extract was diluted with sodium hydroxide, it creates intense yellow color, then addition of Hydrochloric acid it turns to colorless it shows the presence of flavonoids in the sample.
- 4. Tannin : 2 ml of extract added with 2 ml of FeCl3, color was changed into blue to black, it indicates presence of Tannin.
- 5. Anthraquinones (Borntrager's test) : 10 ml benzene added with 1ml of extract and shaken vigorously by filtration then added 5 ml of 10% ammonia and shakes again, it appeared pink/ violet/ red coloration in ammonia layer indicates anthraquinones in sample.
- 6. Glycosides : Ml of extract was hydrolysed by Hydrochloric acid and neutralized by Sodium hydroxide then added equal proportion 1:1 of Fehling solution A and B, it produced red precipitate, shows the presence Glycosides in sample.
- 7. Fatty acid : Extract was added with same amount of ether the it poured on Whatmann filter paper , wait for evaporation of ether, then filter paper seen transparent, it shows presence of fatty acids in sample.

- 8. Triterpenoids: 100 microliter extract was added with 1ml chloroform, 1 ml acetic anhydride and 2ml of concentrated sulfuric acid from sides of the test tube, reddish violet color infers was created , it indicates presence of Triterpenoids in sample.
- 9. Terpenoids (Salkowski test) : 2ml of chloroform added with 0.2 ml of extract then added concentrated sulfuric acid on the side of test tube. It appears reddish brown color at interference indicates the presence of terpenoids.

Fourier-transform infrared spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy, also known FTIR Analysis or FTIR Spectroscopy, this technique is mostly used to analyze biochemical substance as well as identification of organic, inorganic and polymeric materials. In FTIR analysis infrared light scan test sample and checkout various chemical components.

The FTIR instrument sends infrared radiation of about 10,000 to 100 cm-1 through a sample, with some radiation absorbed and some passed through. The absorbed radiation is converted into rotational and/or vibrational energy by the sample molecules. The resulting signal at the detector presents as a spectrum, typically from 4000 cm-1 to 400cm-1, representing a molecular fingerprint of the sample. Each molecule or chemical structure will produce a unique spectral fingerprint, making FTIR analysis a great tool for chemical identification.

Dried powder of *Ganoderma lucidium* finely crushed and mixed with Potassium bromide (Kbr), then put the sample into Shimadzu Infinity Infra Red instrument for analysis of sample.

Antioxidant Assays :

DPPH assay (2, 2-diphenyl-1-picrylhydrazyl)

DPPH is the free radical which is react with antioxidants. In this assay, a molecule or antioxidant with weak A-H bonding will react with a stable free radical DPPH. (2,2-diphenyl-1-picrylhydrazyl, λ max=517 nm) causing discoloration of the molecule. The oxygen radical absorbance capacity assay works based on the measurement of fluorescent signaling by adding fluorescein.

The scavenging rea	ction betv	ween (DPPH) and	an antioxidant (H A)	can be written as
(DPPH) + (HA)	\rightarrow	DPPHH + (A)		
(Purple)		(Yellow)		

In this assay DPPH is used for determined amount of antioxidants present in the extract. Prepared different concentration of extract and add 100µM at equal amount. Incubate reaction for 15 min in dark condition at room temperature. Then absorbance was recorded against blank (Methanol) and control which contained 4ml DPPH and 1ml methanol at 517nm. Reading was taken in triplet.

Calculated the percentage of inhibition by using formula

% of inhibition =
$$AC - AS$$

_____ X 100

AC

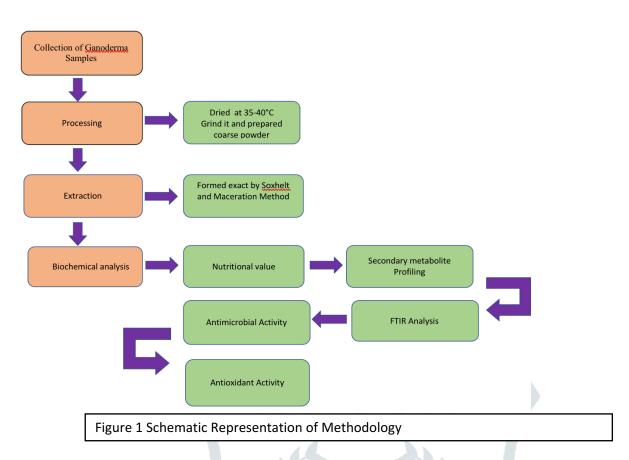
Where, AC is absorbance of control and AS is absorbance of sample. (Fiske et al. 2018).

Antimicrobial Analysis

Selection of microbes:-

Pathogen which is generally affect human such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella pneumoniae* were collected from infected person associated with Department of Microbiology SGBAU Amravati Method of Antibacterial activity by (Well Diffusion Method)

It is very easy Method for analysis of Antibacterial activity. At first prepared Muller Hington agar and properly sterilized in autoclave at 15 lbs for 20 minutes. Then poured media in petriplates at sterile condition (under laminar airflow). Wait for cool. Properly sterilized all instruments like forceps, beakers, distilled water, etc. pour extract on cavity block and dipped Sterile discs and loaded (100 μ l and 150 μ l) sample. Take activity against control and Incubate up to 18 –24 hrs. Then Diameter of zones was calculated in mm. (Baurer et al. 1966).



III. OBSERVATIONS AND RESULTS.

Nutritional value of Ganoderma lucidium

 Table No 1. Nutritional value of 100 gm Powder of Ganoderma lucidum, Ganoderma applantum and Ganoderma multiplicatum.

	Sample 1 (Ganoderma	Sample 2 (Ganoderma	Sample3 (Ganoderma
Contents	lucidium)	multiplicatum)	applantum)
Total Carbohydrates	46.21 %	44.2 %	42.60 %
Proteins	20.64 %	19.25%	20.10 %
Fiber	32.20 %	33 %	32.80 %
Total Ash	2.6 %	2.8 %	2.5 %
Simple Carbohydrates	12.10 %	11.80 %	11.28 %
Reducing Sugar	1.25 %	1.18 %	1.21 %
Total Polysaccharide	29.25 %	29.20 %	28.70 %
Vitamin C	38.40 %	35.40 %	28.50 %

Table No. 2Secondary metabolite profiling of Ganoderma lucidum, Ganoderma applantum and Ganoderma multiplicatum.

 Table No. 2.1 Secondary metabolite analysis in Methanol and Water Solvent System Ganoderma lucidium.

Contents	Methanol	Water
Alkaloid	-	++
Flavonoid	++	+
Tannin & Phenolic compound	+++	+
Antraquinone	+++	++

Glycoside	+++	+
Fatty acids	++	+
Triterpenoids	-	-

Table No.2.2 Secondary metabolite analysis of *Ganoderma applantum* in Acetone, Ethanol, Ethyl acetate and water solvent system.

Metabolites	Ethanol	Acetone	Ethyl Acetate	Water
Cardiac Glycoside	-	-	-	-
Tannin	-	-	-	++
Alkaloid	+++	++	++	-
Leucoanthocynin	-	-	-	-
Non Reducing Sugar	-	-	-	-
Saponin	-		-	-
Terpenoids	++	+++	+++	-
Flavonoids	++		++	+
Glycoside				-
Fatty acids	-		-	-
Triterpenoids	+++	++	+	+

Table No. 2.3 Secondary metabolite analysis of	<mark>Ganoderma m</mark> ultiplicatum	in Acetone, Ethanol, Ethyl acetate and water
solvent.		

solvent.				
Metabolites	Ethanol	Acetone	Ethyl Acetate	Water
Cardiac Glycoside		-		-
Tannin		-	6-7	-
Alkaloid	++	-		++
Leucoanthocynin	-		-	-
Non Reducing Sugar	-	-	-	-
Saponin	-		-	-
Terpenoids	+++	+++	++	-
Flavonoids	++	-	+++	++
Glycoside	-	-	-	-
Fatty acids	-	-	-	-
Triterpenoids	++	+	+	+

Table No. 3 FTIR Analysis of Ganoderma lucidum, Ganoderma applantum and Ganoderma multiplicatum.

Table No. 3.1 FTIR Analysis of Ganoderma lucidum

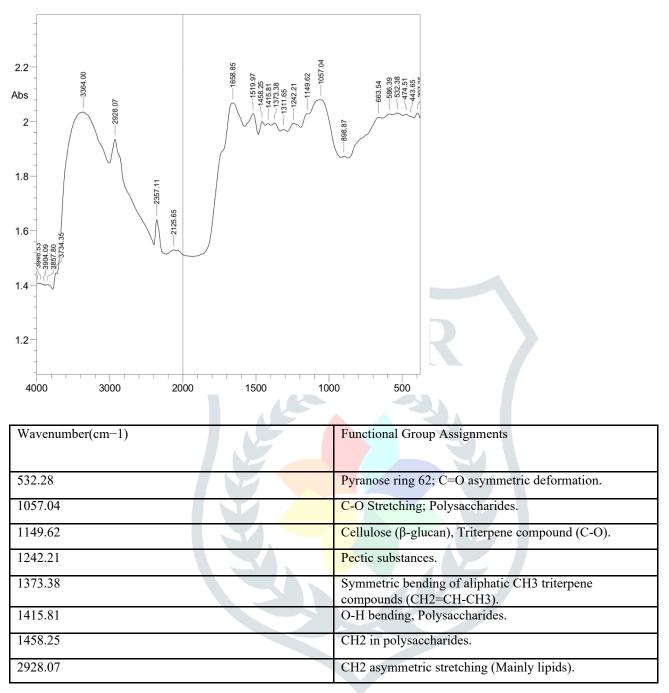


Table No. 3.2 FTIR Analysis of Ganoderma multiplicatum

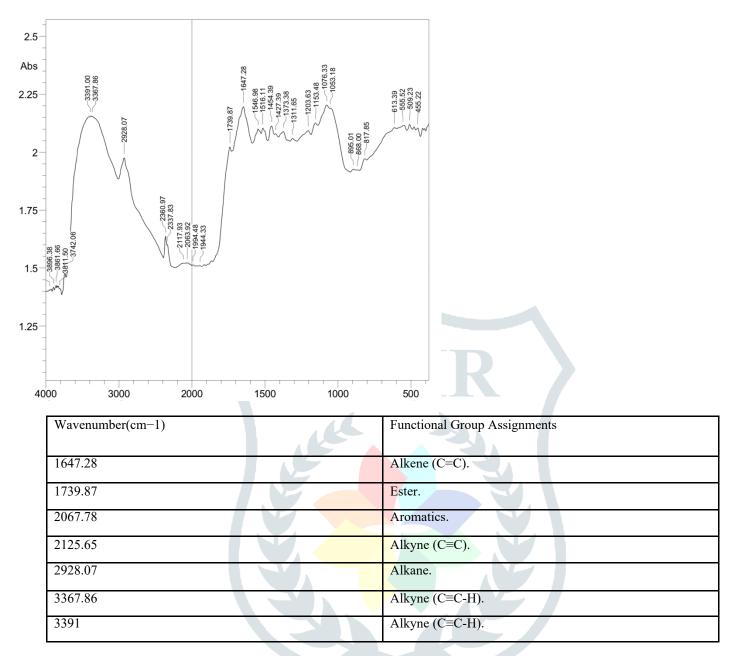


Table No. 3.3 FTIR Analysis of Ganoderma applantum

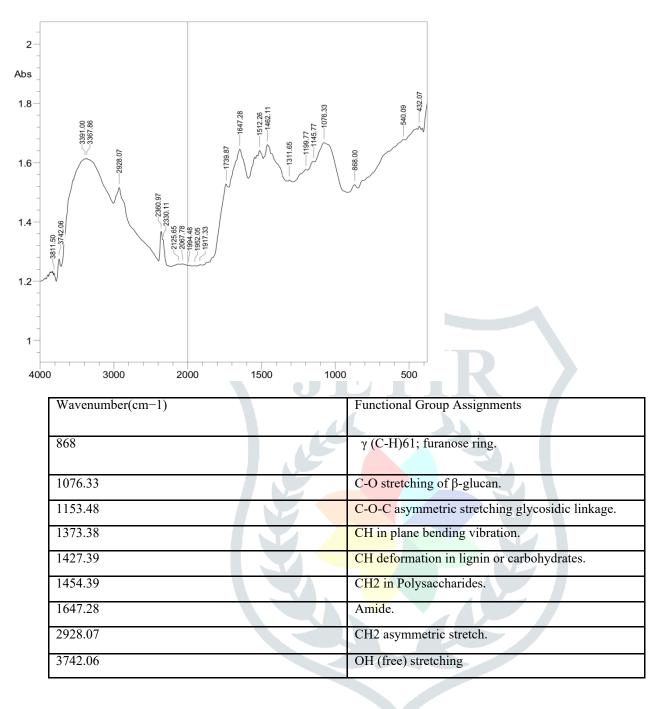


Table No. 4 Antioxidants activity (%) of Ganoderma lucidum, Ganoderma applantum and Ganoderma multiplicatum.

DPPH RSA%
87.671 %
82.296
53.249

Antimicrobial Analysis

 Table No. 5 Antibacterial Analysis against Gram positive and Gram negative.

Table No. 5.1 Antibacterial activity of *Ganoderma lucidium*(Graph No. 4.2)

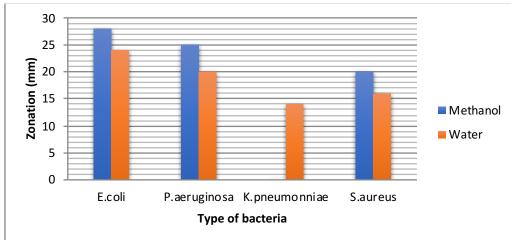
Solvent / Bacteria	Methanol	Water
Escherichia Coli	28 mm	24 mm
Pseudomonas aeruginosa	25 mm	20 mm
Klebsiella pneumoniae	NA	14 mm
Staphylococcus aureus	20 mm	16mm

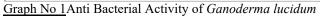
Table No. 5.2 Antibacterial activity of Ganoderma applantum. (Graph No. 4.3)

Solvent / Bacteria	Ethanol	Acetone	Ethyl Acetate	Water
Escherichia Coli	20 mm	17 mm	22 mm	15 mm
Pseudomonas aeruginosa	12 mm	NA	12 mm	15 mm
Klebsiella pneumoniae	NA	22 mm	NA	14 mm
Staphylococcus aureus	20 mm	22 mm	15 mm	NA

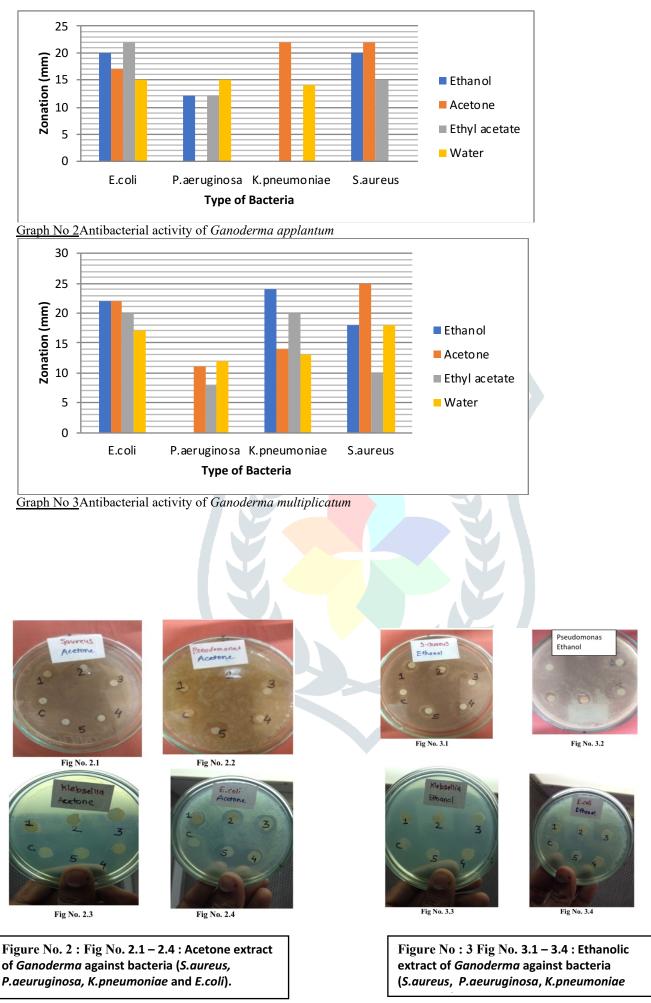
Table No. 5.3 Antibacterial activity of Ganoderma multiplicatum(Graph No. 4.4)

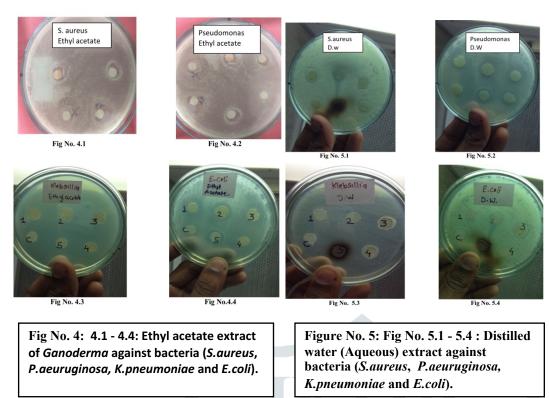
Solvent / Bacteria	Ethanol	Acetone	Ethyl Acetate	Water
Escherichia Coli	22 mm	22 mm	20 mm	17 mm
Pseudomonas aeruginosa	NA	11 mm	8 mm	12 mm
Klebsiella pneumoniae	24 mm	14 mm	20 mm	13 mm
Staphylococcus aureus	18 mm	25 mm	10 mm	18 mm











In All Figures – (C) Represents Control, (1) Represents Sample of *G. lucidium*, (2) Represents Sample of *G. applantum*, (3) Represents Sample of *G. multiplicatum*. It represents anti microbial activity against gram positive bacteria (*S. aureus*) and Gram negative bacteria (*Pseudomonas, Klebsillia and E.coli*).

Ganoderma has medicinal as well as nutritional fungi in nature. It shows massive amount of medicinal components which is essential for human body. *Ganoderma* shows nutritional value (Table 1) various nutritional compounds such carbohydrates, Proteins, Fiber and various other compounds are present in *Ganoderma*. But *Ganoderma lucidum* shows higher amount of nutritional value as compare to *Ganoderma applantum* and *Ganoderma multiplicatum*. So it must be helpful for human body. As same as secondary metabolites Alkaloids, Triterpenoids, Glycosides, Phenolic compounds, flavonoids also mostly present in specially *Ganoderma lucidum* (Table 2). Various functional group is detected in FTIR analysis (Table 3). Antioxidant plays tremendous role for human body. Dozens of diseases are caused due to free radicals. today's our envoimental condition are more polluted and that is the source of free radicals. Antioxidant is most beneficial and demanding food so *Ganoderma lucidium* shows 87 percent of scavenging activity against the DPPH free radicals (Table 4). There are various disease causing due bacteria called as bacterial infection. Infection caused by *E.coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus* May be cure by this three species of *Ganoderma* all this three species shows strongly activity against *E. coli, P. aeruginosa and S.aureus* in Methanol Solvent. *Ganoderma applantum* shows strongly activity against *E. coli*, *P. aeruginosa and S.aureus* in Methanol Solvent. *Klebsiella pneumonia* in Ethanol and *Ganoderma multiplicatum* shows strongly activity against *E. coli* in Ethyl Acetate Solvent, *Klebsiella pneumoniae* and *Staphylococcus aureus* in Acetone Solvent and *Ganoderma multiplicatum* shows strongly activity against *E. coli* in Ethanol and Acetone Solvent. *Klebsiella pneumonia* in Ethanol and ethyl acetate Solvent and *S.aureus* in Acetone Solvent system (Graph 1-3).

Conclusion

Melghat is beauty of central India zone. But sometimes all things are not present at one place. Malnutrition is very huge issue of Melghat region. Various commissions are try to remove Malnutrition. Most Victims of Malnutrition are in Melghat region. Numbers of peoples face this overcoming problem. Data shows that rate of stunting, wasting and underweight issue. So it is not good for society. Various funds and techniques was already used for this issue. For resolve this issue try some different type food material which is having nutritional as well as nutraceutical values. Because all problems are not solved by only nutritional food. due to lack of nutrition faces various problem. Such as eye problems, Heart problem, weak immunity and so on. So they require most nutritional and nutraceutical food which is rapidly recovers their health. Due to weak immunity system foreign pathogens may be easily attack and that may take more time to recover. *Ganoderma lucidum* containing nutritional value, medicinal, Antioxidants, Antibacterial and so many various properties are present. So this types food material which is Healthy, medicinal and easily available in that region may be helpful for them.

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