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Comparative Physico-Chemical, Phytochemical, GC - MS and Antibacterial Studies between Ethanolic Extract of Fresh Green Leaves and Sun Dried Leaves of Tilkor (*Momoradica monadelpha*)

Panshu Pratik[1*] and Ram Vinay Thakur[2]

[1] Dept. of Chemistry, M.L.S.M. College, L.N.M.U., Darbhanga, Bihar, INDIA
[2] Univ. Dept. of Chemistry, L.N.M.U. Darbhanga Bihar, INDIA

[*] Correspondence: E-mail: pratikpanshu@gmail.com

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ABSTRACT: In continuation to our large number of previous works on Tlikor and keeping this view in our mind that "Baids" often prefers fresh green leaves of medicinal plants for their herbal preparation, in this paper, works has been carried out as per intention to high light the comparative studies between ethanolic extract of fresh green leaves and sun dried leaves of Tilkor in terms of their Physico chemical studies, phytochemical investigation, GC–MS analysis and antimicrobial examinations.

Keywords: *Momoradica monadelpha*; Tilkor; Physicochemical analysis; Phytochemical investigation and antimicrobial study.

INTRODUCTION: Plants are considered to be the life line for human beings as it is essentially needed in our life for most of the needful things such as foods, clothes & shelters. Further their utility for the treatment of various kind of diseases was also being revealed.[1] Then these plants were started to use for the purpose of treatment in all over the world & are considered as medicinal plant.[2] Viewing the utility of plant as medicine, several research were carried out in order to study and characterize the chemical components present in the plants.[3-14]

Thereafter different methodologies for standardization of plant, extraction, isolation, identification, characterization, phytochemical & physicochemical investigation as well as pharmacological assay was also been developed.[15] And with gradual passage of time, standarsation report of plants come into the existence,[16-18] which were considered to be the greatest contribution to the society due to their revealed pharmacological effect. The characterization report were instigated several scientist for synthesis drug design & discovery and also the vital role of different metal in biological purpose were come into the existence.[19]

Tilkor also known by its biological name Momoradica Monadelpha is consider to be one of the most traditional plant of Mithilanchal (Darbhanga), Bihar India and is employed for preparation of several types of dishes as well as for medicinal purpose too.
The plant is naturally grown in India and having very high nutritive as well as medicinal value. In practice of baids it is being used for the treatment of diabatese, lever problems, immunity, osteoarthritis, joint pains etc. It has also antinaphylatic, anti hyperglycemic and anti oxidant properties. But inspite of this most of the pharmacological activities of the plant are not yet established by the clinical trial. However all the significant results related to this plant, which are reported in literature are belongs to my previous research works and are summarized as:
In the methanolic extract of leaves of this plant, phytochemicals like terpenoids, steroids, tannuins, alkalic flavonoids & tammins are present. The characterization report of the isolated components from the extract revealed the presence of compound stigmosterol and tritiacontane in it. They have also found to have anti microbial activity.[20]

Methanolic extract of flower of this plant contain phytochemicals like phenol, acid carbohydrate flavonoid, saponin alkacoid, tammins, cardiac glycoside, protein, lipids and steroids. They also have antimicrobial activity and the structure of the isolated compounds from the extract revealed as n-hexadecanyl oleate and n-dotricontane -15-one.[21]

The ethanolic extract of fruit of this plant showed the presence of phytochemicals like acid, lipid, flavonoids, alkaloids, tannins saponins, cardiac glycosides, phenol, proteins & carbohydrate. The compounds which are isolated from the extract of fruit are characterized as: β-sitosterol-3-o-B-D-glycoside and n-

tetracontane -7-one. They also found to have antimicrobial activity.[22]

Mucilage of fruit of this plant has fats, proteins, phytosterols & carbohydrate. The Mucilage also posses an antimicrobial activity which is found to be very effective against staphylococcus anreus. The isolated compound from mucilage was characterized as Quercetin.[23]

Among the antibacterial activity of leaf steam & root of this plant in methanolic extract against E.coli, S. Typhi & S. Aureus, the order of activity of plant's part are found to be as Root > Leaf > Steam and order of sensitivity of bacterial species was found to be as S. Typhi > S. Aureus > E. coli.[24]

So expansion of research works on this plant is seems to be very much essential. Therefore in order to reveal the mystery behind the maximum pharmacological efficacy of fresh green leaves of this plant in comparison to sun dried leaves, the present work is totally focused on comparative study between ehanolic extract of fresh green leaves and sun dried leaves of this plant in terms of its physicochemical and phytochemical investigations, identification GC-MS analysis and pharmacological activities with reference to antimicrobial activity.

MATERIALS AND METHODS:

Chemical & Instruments: All analytical grade solvents and chemicals are being used without any purification such as ethanol, silica gel, n-hexane, chloroform, sulphuric acid, Mayer's reagent, hydrochloric acid, magnesium ribbon, acetic anhydride, dil sodium hydroxide, DMSO etc. and a Soxhlet extractor, T.L.C. plates and Tray, incubated plates as well as incubation machine were used as an instrument.

Plant Material: The leaves of Tilkor [*Momoradica monadelpha*] were collected from my own garden and it was then verified by the Eminent Botanist Prof. Shashi Shekhar N. Sinha [International scientist of Radiation genetics].

Extraction of leaves: The extraction of both fresh green and some dried leaves of plant were carried out by Soxhlet extraction method by using Soxhlet extractor [914/7] and by using ethanol as solvent.

Physico-chemical parameters & phytochemical screening: Determination of physico-chemical parameters were carried out by guidelines provided by WHO[25] and the quantitative & qualitative phytochemical screening were carried out by the separated protocol.[26-29]

Separation of components in the ethanolic extract of both the sun dry & green leaves was carried out by Thin Layer chromatography. It was perform by the help of Glass plate coated by silica gel by standard method.[30]

Antimicrobial Assay: Agar well diffusion method (NCCL. B, 1995) was used for determining antimicrobial activity of ethanolic extract of both green and dried leaves of plant. A natural agar was used for growing bacterial strain. Here the plant extract was dissolved in DMSO to prepared the solution at conc. 25, 50, 100, 200 ml/mg. 40 mg/ml solution of levofloxacin was taken as reference antibiotic.

GC- MS Analysis: GC-MS analysis of ethanolic extract of both green and dried leaves of Tilkor were performed with Agilent 1909 1Gc plus automatic sampler system coupled with mass spectro meter 433 HP – 5 MS [at Rajendra Agriculture University, Pusa, Bihar India.]

Physicochemical parameters: pH of 15% & 30% ehanolic extract of both green & dried leaves was determined by calibrated digital pH meter and other physicochemical parameters were determined by standard method.[31 to 33]

Phytochemical Investigation: Quantitative and Qualitative Phytochemical investigations of ethanolic extract of both fresh green & dried leaves of the plant were done by standard experimental tests.[18, 20, 29, 33]

Extraction: The ethanolic extract of both powdered dried leaves & green leaves was carried out by Soxhlet extractor by using ethanol as solvent [by standard method].

Separation of components: Ethanolic extract of both types of leaves were taken in two separate 200 ml beaker and then strried well and filter are well as evaporated by rotary evaporator. Now by using silica gel [GF024391] for the purpose of absorbent and ethanol as solvent, the T.L.C. finger print profile has been developed.

Pharmacological activity [Antimicrobial activity]: The requisite pathogenic bacteria & fungi were collected from Medical College of Darbhanga [Microbiology department] viz. prevotella intermedia [ATCC (225)] Porphyromonas giugiraus [ATCC (33658)] Aggregatibacter actinomyces emconitians [ATCC (12745)] sligella shigella [ATCC (94295)] and staphylococcus aure bls [ATCC(10835)] and are tested in 7 mg of Nutrient agar / 300 ml. The slants are incubated at 50° c for 50 hour & are stored at 6° C.

The inculums adjusted at 550 μm leading to transition equivalent to 1 x 10 cell/m. The powdered leaves of both types were dissolved in DMSO and levofloxacine was taken as reference antibiotic. All the plates

were incubated with 30 μ g/ml Microbial suspension having concentration of 1 x 10^8 cell.

GC-MS analysis: Compounds were separated in HPSMS column fused with phenyl ethyl silox. [Length 35 m x 260µm] film thickness 0.30 µm) samples were injected at a temperature of about 275° C with ratio of split 15:5 and rate of flow of helium is 2 ml/min. Extract of both green & dried leaves of Tilkor were dissolved in the solvent ethanol to form a solution. Thereafter the extracts were put into GC-MS instrument for the respective constituent and their mass spectra were obtained.

RESULTS AND DISCUSSION:

Results:

Physico-chemical analysis: The comparative physico chemical analysis of sample of both fresh green leaves and shade dried leaves are given in Table - 1.

Table 1: Comparative results of various physico chemical parameters of sample of both green & dried leaves of plant.

S. No.	Parameters	Results of Green leaves	Results of dried leaves
1.	Total ash	17.85 % of green wt.	11.85% of dry wt.
2.	Moisture content	1.19%	0.58%
3.	Acid insoluble ash	1.12% of total ash	2.45% of total ash
4.	Water soluble ash	5.02% of total ash	3.45% of total ash
5.	Alcohol soluble extraction	18.2% of green wt.	9.3% of dry wt.
6.	Water soluble extraction	16.92% of green wt.	6.85% of dry wt.
7.	pH of 15% solution of ethanolic extract of plant	7.31	5.04
8.	pH of 30% solution of ethanolic extract of plant	6.80	4.77

Phytochemical Investigations: The comparative phytochemical investigations of ethanolic extract of both fresh green and sun dried leaves of plant are given in Table 2.

Table 2: Comparative results of various phytochemical parameters of ethanolic extract of green and dried leaves of plant are given.

S. No.	Phyto-chemical	Test's Name	Result of ethanolic extract of green leaves	Result of ethanolic extract of dried leaves
1.	Flavanoid	1. Alkaline regent test 2. Shinoda test	+++ +++	- -
2.	Steroid	H_2SO_4 Test	++	+
3.	Saponins	Foam Test	++	++
4.	Terpero-nids	Liberman's Test	++	-
5.	Tannins	$FeCl_3$ Test	++	-
6.	Aklaloids	Mayer's Test	+++	++
7.	Cardiac glyco-sides	Kellar Kilai-ni Test	+	-

Where; + *Indicates present*
- *Indicates absent*
+++ *Indicate highest degree of precipitation*
++ *Indicate moderate degree of precipitation*
+ *Indicate lowest degree of precipitation*

Table 3: Comparative quantitative phytochemical investigations of both dried and green leaves.

S. No.	Phytochemical	Amount in green leaves extract	Amount in dried leaves extract
1	Flavonoid (mg/100 gm)	20.05	00.00
2.	Steroid (mg/100 gm)	35.05	34.08
3.	Saponins (mg/100 gm)	13.07	12.05
4.	Terpenoids (mg/100 gm)	18.08	00.00
5.	Tannins (mg/100 gm)	3.79	00.00
6.	Alkaloids (mg/100 gm)	1.15	1.14
7.	Cardiac glyco-side (mg/100 gm)	2.24	00.00
8.	Waste chemicals	6.57	52.73

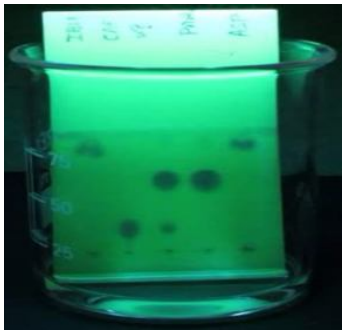


Figure 1: TLC Plate of Fresh Green Leaves.

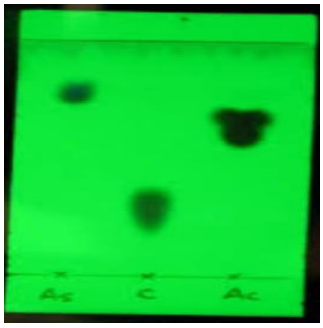


Figure 2: TLC Plate of Sundried Leaves.

Thin Layer Chromatography (TLC): Finger print profile of TLC for ehanolic extract of both types of

leaves was developed by using chloroform, methanol & n – hexane in the ratio 2.0: 4.0: 6.0(v/v/v). 6 Spots were observed for ethanolic extract of fresh green leaves of plant (figure1) and 3 spots were observed for methanolic extract of dried leaves of plant (figure 2) under UV (of 360 nm) light when visuallised by using vanillin sulphuric acid.

Table 4: Comparative Antibacterial Study of Fresh Green Leaves and Sun Dried Leaves.

S. No.	Organism	Ethanolic Extract of Fresh green Leaves					Ethanolic Extract of dried Leaves				
		Zone of inhibitation (nm) in conc. mg/ml				Reference Antibiotic	Zone of inhibitation (nm) in conc. mg/ml				Reference Antibiotic
		25.00	50.00	100.00	200.0	40mg/ml	25.00	50.00	100.00	200.00	40mg/ml
1	Prevotella Intermedia	–	–	–	9.5	14.0	–	–	–	9.1	13.8
2	Porphyromonas gingivalis	–	–	8.4	10.5	15.3	–	–	8.2	10.2	15.0
3	Aggregati bacter acteno mycemco means	–	–	–	8.1	11.2	–	–	–	7.9	10.8
4	Shigella shigel-la	–	–	–	8.5	12.3	–	–	–	8.1	12.0
5	Staphylococcus aureus	–	–	13.5	15.2	13.0	–	–	13.1	14.9	12.7

Table 5: GC- MS analysis of Ethanolic extract of green leaves of Tilkor.

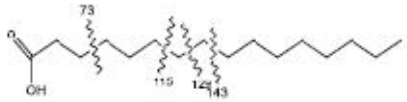
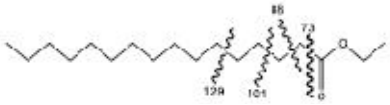
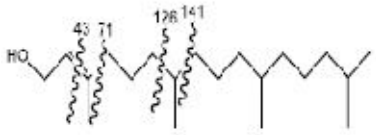
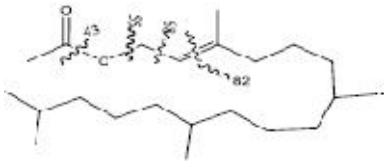
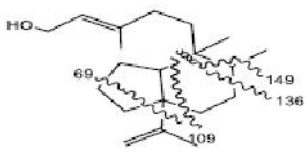
S. No.	Compound	Molecular formula	MW	Peak area %	Retention Time (min)	Mass spec-tral frag-ments	Fragmented structures
1.	Palmitic acid	$C_{16}H_{32}O_2$	256	9.80	16.782	43, 60, 85, 98, 73	
2.	exadecanoic, ethyl ester	$C_{18}H_{36}O_2$	284	10.89	17.048	57,73,101, 115, 88	
3.	Phytol	$C_{20}H_{40}O$	29 6	7.22	18.304	57, 95, 111, 71	
4.	Phytol acetate	$C_{22}H_{42}O_2$	338	6.19	19.305	43, 82, 95, 123, 68	
5.	5-(7a- Iso-propenyl - 4,5-dimethyl-octahydroinde n-4-yl)-3-methyl-pent-2- en-1-ol	$C_{20}H_{34}O$	290	4.93	22.049	81, 95, 109, 123, 149	

Figure 3: Incubated Plates of Fresh Green Leaves.

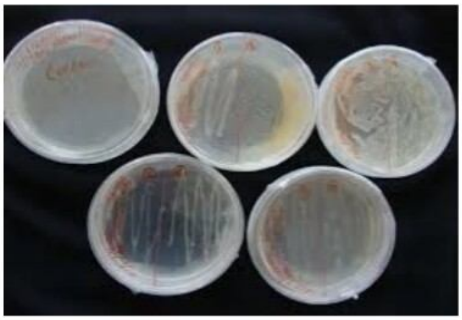


Figure 4: Incubated Plates of Dried Leaves.

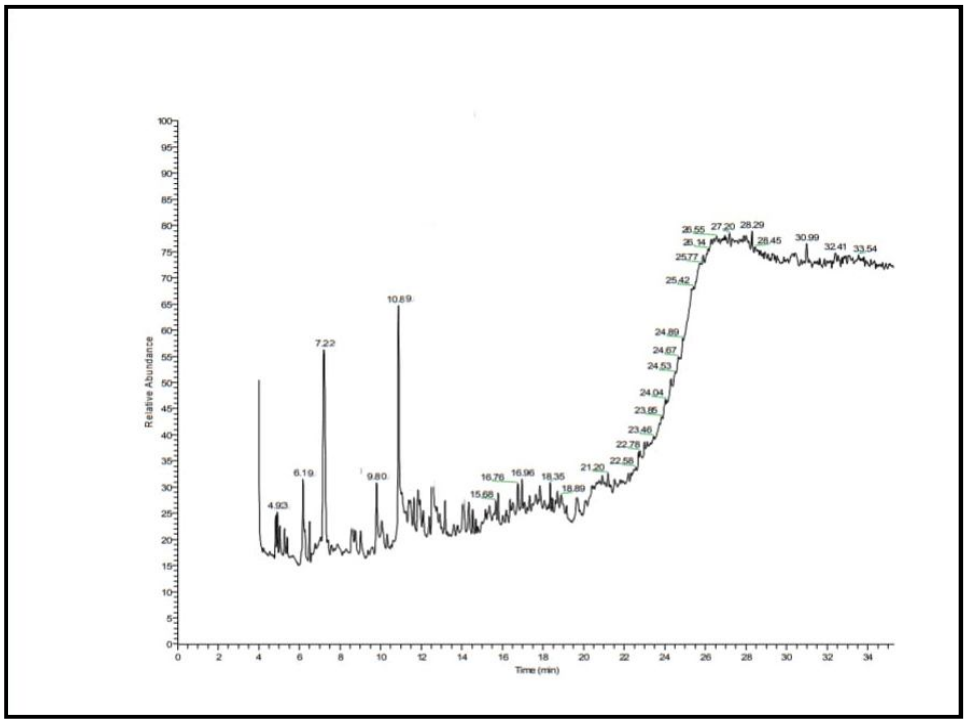
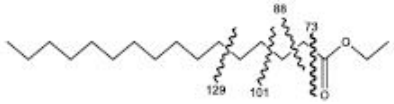
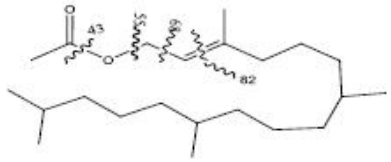
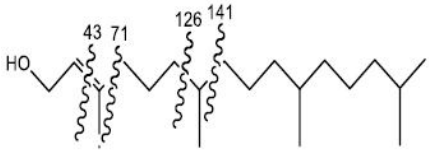


Figure 5: Chromatograph of Fresh Green Leaves.

Table 6: GC- MS analysis of Ethanolic extract of dried leaves of Tilkor.

S. No.	Compound	Molecular formula	MW	Peak area %	Retention Time (min)	Mass spectral fragments	Fragmented structures
1	Exadecanoic, ethyl ester	$C_{18}H_{36}O_2$	284	10.89	17.048	57,73,101, 115, 88	
2	Phytol acetate	$C_{22}H_{42}O_2$	338	6.19	19.305	43, 82, 95, 123, 68	
3.	Phytol	$C_{20}H_{40}O$	296	7.22	18.304	57, 95, 111, 71	

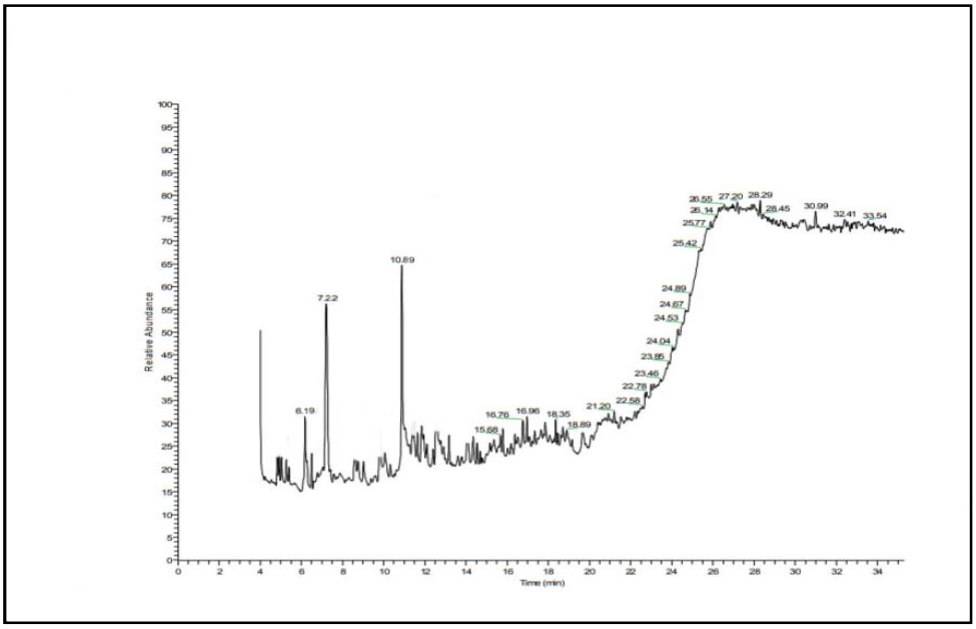


Figure 6: Chromatograph of Dried Leaves.

Discussion: The comparative results of physico chemical parameters of sample of both fresh green leaves and sun dried leaves of Tilkor were given in Table 1.

In the sample of fresh green leaves, the % of total ash is 17.85%, moisture content is 1.19%, acid insoluble extractive is 1.12% water soluble ash 5.02%, alcohol soluble extractive 18.2% , water soluble extractive 16.92% an pH (15% extract) is 7.31 and pH(30% extract) 6.80. All the parameters of fresh green leaves sample are found to be greater in comparison to the parameters of sample of sun dried leaves. i.e. Total ash 11.8%, moisture content 0.58% and insoluble ash 2.45%, water soluble ash 3.45%. Alcohol soluble extractive 9.3% water soluble extractive 6.85% pH (15% extract) 5.04 & pH (30%) 4.77.

Qualitative phyto-chemical investigations of ethanolic extract of fresh green leaves revealed the presence of seven phyto-chemicals viz. – Flavonoids, steroid, saponins, Tannins, Terpenoids, Alkaloids and cardiac glycosides. In which Flavonoids and alkaloids showed highest degree of precipitation (+++), cardiac glycoside showed lowest degree of precipitation (+) and rest – showed moderate degree of precipitation (++). However ethanolic extract of dried leaves revealed the presence of only three phytochemicals alkaloids, saponins and steroid in which degree of precipitation of first two is moderate while third is lowest. (Table 2)

Quantitative phytochemical investigation of green leaves extract revealed the amount of phytochemicals (in mg/100gm) as 20.05 mg flavanoid, 35.05 mg steroid, 13.07 mg saponins, 18.08 mg terpenoids, 3.79 tannins, 1.15 mg alkaloids, 2.24 mg cardiac glycosides & waste dead chemicals 6.57 mg. However in dried leaves extract only 3 phytochemicals are present in amount of steroids 34.08 mg saponins 12.05 mg and alkaloids 1.14 mg along with death waste chemicals 52.73 mg. (Table 3)

Separation of component using thin layer chromatography when visualized under UV light then showed six spots in green leaves extract but only three spots in dried leaves extract. (Figures 1 & 2).

Antimicrobial examination of ethanolic extract of fresh green leaves showed higher antimicrobial activities at 200 mg/ml conc. extract against Staphylococcus aureus 15.2 nm in comparison to reference antibiotic (13.0 nm) and ethanolic extract of dried leaves also showed the same against same bacteria at 200mg/ml by 14.9 nm in comparison to the reference antibiotic 12.07 nm. But antimicrobial activities of dried leaves against all the rest bacterias were found to be lesser than that of green leaves (Table 4, Figures 3 & 4).

GC-Ms analysis of ethanolic extract of fresh green leaves showed the presence of five component viz. – Palmitic acid, Hexadecanoic ethyl ester, Phytol, Phytol acetate and 5-(7a-isopropenyl) 4, 5- dimethyl-octohydrnude n-4-yl)-3-methyl-pent-2-en-1-ol at peak area 9.80, 10.89, 7.22, 6.19 and 4.93 respectively in which most abundant one is hexadecanoic ethyl ester (at peak area 10.89) (Table 5, Figure 5). However Gc-Ms analysis of dried leaves showed the presence of only three component viz. Hexadecanose ethyl ester, phytol acetate and phytol at peak area 10.89, 6.19 and 7.22 respectively, in which most abundant one is again hexadecanoic ethyl ester (at peak area 10.89). (Table 6, Figure 6).

CONCLUSION: From this research work it can easily be concluded that the extract of fresh green leaves of plant is more efficient than sun dried leaves of plant in terms of their pharmacological activities, physico chemical parameters, quantitative and qualitative phytochemicals investigations antimicrobial examination, GC-MS and TLC analysis.

In physiocochemical parameter, the % of different parameters in dried leaves is lesser than green leaves. The same result has been observed in quatitive phytochemical investigations.

Qualitative phytochemical investigation revealed the presence of seven phytochemical in green leaves but only three phytochemical were present in dried leaves extract of the same plant.

TLC of green leaves extract showed six spots i.e. presence of six component but only 3 sports were observed in TLC of dried leaves extract.

GC-MS analysis showed total five component in extract of fresh green leaves but showed only three components in extract of dried leaves.

The anti microbial activities of extract of dried leaves against all five bacterias are much lesser that of extract of fresh green leaves.

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