Pharmacognostical studies, antioxidant activity and cyclophosphamide induced alopecia treatment by *Ziziphus nummularia* (Burm. f.)Wight & Arn. leaves extracts

Sonia and Sumitra Singh*

Department of Pharmaceutical Sciences, Guru Jambheshwar University of Sciences and Technology, Hisar, Haryana, India

Corresponding author:

Dr. Sumitra Singh

Professor

Department of Pharmaceutical Sciences,

Guru Jambheshwar University of Sciences and

Technology, Hisar-125001,

Haryana, India

Email: sumitra.singh32@gmail.com

Abbreviations: α, α-diphenyl-β-picryl-hydrazyl-DPPH; Hair follicle-HF; Anagen: Telogen ratio-A/T; Cyclophosphamide induced alopecia- CIA; Epidermal growth factor- EGF, Insulin like growth factor-I (IGF-I).

Abstract: The main aim of present study was to establish pharmacognostical parameters and to evaluate antioxidant potential and anti-alopecic activity of *Ziziphus nummularia* (Burm. f.) Wight &Arn. leaves n-hexane and hydroalcoholic extracts. The plant leaves were analysed for macro and, microscopical studies, physico-chemical parameters, fluorescence studies, elemental analysis, quantitative estimation of total phenolic content, flavonoid content, tannin content, total saponin, alkaloid content. Antioxidant potential and IC_{50} value was studied by using different assays. Cyclophosphamide induced alopecia model was used in Swiss albino mice for anti-alopecic activity and minoxidil was used as standard drug. Monitoring parameters were hair follicular density, anagen:telogen ratio and histological observation of treated animal skin sections. Both the tested extracts showed antioxidant potential, however hydroalcoholic extract showed better antioxidant activity than n-hexane leaves extract. n- hexane and hydroalcoholic leaves extracts revealed anti-alopecic activity of *Ziziphus nummularia* but hydroalcoholic extract showed better anti-alopecic activity as compare to n-hexane extract. Pharmacognostical studies will help to establish quality standards, purity and sample identification of *Z. nummularia* plant leaves. The results of antioxidant and anti-alopecic activity would help to prove medicinal effectiveness of these extracts.

Keywords: *Ziziphus nummularia* (Burm. f.) Wight &Arn., antioxidant activity, cyclophosphamide, alopecia, histopathological.

1. INTRODUCTION: *Z. nummularia* is a bushy much branched shrub 1-2 m in height with zigzag branches. Leaves are tiny, circular or ovate to elliptic, dark green in colour, margins entire or toothed and base is round

(Rathore M, 2009). *Z. nummlaria* used in treatment of mental retardation, preventing frequent attacks of influenza, colds, treating dysentery, diarrhoea and colic (Kumar *et al*., 2011). Alopecia is one of the most psychological devastating aspects of cancer therapy (Macquart-Moulin et al., 1997; Valdimir, 2003). Till date, no satisfactory treatment for suppressing chemotherapy-induced alopecia in man. Many growth factor families are involved in hair follicle cycling like fibroblast growth factor, epidermal growth factor (EGF), hepatocyte growth factor, insulin like growth factor-I (IGF-I), Transdermal growth factor-β (TGF-β) families (Peus and Pittelkow, 1996). Cyclophosphamide is used an anti- cancerous drug and it causes induction of alopecia because of follicle dystrophy and premature induction of follicle in growing hair follicles (Patel et al., 2014). Synthetic drug like minoxidil is being used for the treatment of alopecia (Batchelor, 2001). But use of synthetic drugs are associated with many adverse effects thus to reduce side effects drugs from natural origin are necessary to replace them. The main goal of present study is to report antioxidant activity and treatment of cyclophosphamide induced alopecia by using *Z. nummularia* leaves extracts.

2. MATERIAL AND METHODS

2.1 Plant material

Ziziphus nummularia plants were collected from open fields of Karnal, Haryana in month of July, 2016. The plant was authenticated as *Ziziphus nummularia* (Burm. f.) Wight & Arn. family Rhamnaceae by Dr. Anjula Pandey, Principal scientist, National Herbarium of cultivated plants, NBGPR, New Delhi, vide reference no, NHCP/NBGPR/2016-12 dated 19.08.2016. The leaves of the plant were selected for the proposed work. The leaves were dried under shade and coarsely powdered for further study.

2.2 Drugs and chemicals

α, α-diphenyl-β-picryl-hydrazyl, methanol, ascorbic acid, citric acid, potassium ferricyanide, trichloroacetic acid, ferric chloride, hydrogen peroxide, ferrozine. Minoxidil (MP Biomedicals, Maharashtra, India), cyclophosphamide (Himedia laboratories, Mumbai, India), n-hexane (High Purity Laboratory Chemicals, Gujarat, India) and ethanol (Loba chemicals, Mumbai) were purchased. All the chemicals were of analytical grade.

2.3 Preparation of plant extracts

The air-dried leaves powder of *Z. nummularia* was extracted with n-hexane and hydroalcoholic by using soxhlet apparatus. Extracts were evaporated to dryness to obtain dried extracts and were kept in desiccator. The percentage yield of n-hexane and hydroalcoholic extracts were 3.5±0.6 and 5.02±0.12 % w/w respectively. Both extracts were further subjected to various activities.

2.4 Pharmacognostical study of *Z. nummularia* leaves

2.4.1 Macroscopy

For macroscopical studies fresh leaves of *Z. nummularia* were performed by visual examination (Pandaya et al., 2010; Khandelwal, 2008).

2.4.2 Microscopy

Coarse powder of leaves were used to study microscopical characters. For the microscopical studies, transverse sections of leaves were prepared and stained accordingly. The powder microscopy was performed according procedures (Pandaya et al., 2010; Khandelwal, 2008).

2.4.3 Physicochemical analysis

The physico-chemical parameters such as percentage of total ash, acid-insoluble, water soluble and sulphated ash, loss on drying, extractive values, foaming index, swelling index, crude fiber content, haemolytic activity, foreign organic matter, bitterness value, microbiological and pathogen analysis were determined according to official methods for quality control of *Z. nummularia* plant leaves (Mukherjee, 2002; Anonymous, 2004; WHO, 2007; Singh and Sonia, 2018).

2.4.4 Fluorescence analysis: Powdered leaf material of *Z. nummularia* were treated with various chemical reagents and exposed to viewed in day light, short (254 nm) and long and (365 nm) ultraviolet radiations. The colors observed by application of different reagents in different radiations were recorded (Pratt and Chase, 1949).

2.4.5 Elemental analysis: Elemental analysis of *Z. nummularia* leaf powder was done using nitric-perchloric acid digestion method using the procedure recommended by the AOAC (1990).

2.4.6 Quantitative phytochemical study

Determination of total phenol content

The level of total phenols in hydroalcoholic extract of leaves powder extract was determined by using Folin–Ciocalteu reagent and external calibration with gallic acid (Singleton et al., 1999; Singh et al., 2012).

Determination of flavonoid content

The flavonoid content of hydroalcoholic extract of dried leaves powder extract was determined by aluminium chloride method and quercetin used for external calibration (Singh et al., 2012).

Determination of tannin content

Folin - Ciocalteu method was used for determination of tannins (Singh et al., 2012).

Determination of saponin content

The determination of total saponin was done by standard method (Theresa Ibibia Edewr et al., 2016).

Determination of alkaloid content

Shamsa et al., 2008 method was used for determining total alkaloid content (Manjunath et al., 2012).

2.5 *In-vitro* antioxidant activity

Antioxidant activities of *Z. nummularia* leaves extracts was determined by using various assays like α, α-diphenyl-β-picryl-hydrazyl (DPPH) free radical scavenging activity, reducing power (Arumugam and Venugopal, 2016; Roduan et al., 2019), hydrogen peroxide (H_2O_2) scavenging activity (Sroka and Cisowski, 2003) and metal chelation activity (Soler-Rivas et al., 2000).The IC_{50} was calculated, it is a parameter widely used to measure the antioxidant activity of test samples. It is the concentration of the sample that could scavenge 50% free radical (Sanchez-Moreno et al., 1998; Rivero-Cruz et al., 2020).

2.6 Cyclophosphamide induced alopecia

2.6.1 Test solutions

Cyclophosphamide (50 mg/kg, i.p.) dissolved in distilled water. n-hexane and hydroalcoholic extracts solution were prepared in ethanol: propylene glycol: water (8:1:1).

2.6.2 Animals

Swiss albino mice (20-25gm in weight), 3-4 months old, 24 either sex were obtainedfrom Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences Hisar, India. The experimental protocol was approved by the Institutional Animals Ethics Committee (CPCSEA/436/PO/Re/S/2001, 20-06-2016) and care of laboratory animals was taken as per guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forests and Climate Change, Govt. of India, New Delhi.

2.6.3 Grouping of animals

The animals were randomly divided in 4 groups of 6 Swiss albino mice in each group, cyclophosphamide (50 mg/kg, i.p.) the inducer was injected in all groups after 9^{th} day of treatment.

Group 1 (control) was treated with vehicle (ethanol: propylene glycol: water in 8:1:1) topically; group 2 (standard) was treated with minoxidil (5% w/v in ethanol) topically; group 3 (test) treated with *Z. nummularia* leaves n- hexane extract (50% w/v) topically and group 4 (test) treated with *Z. nummularia* leaves hydroalcoholic extract (50% w/v) topically.

2.6.4 Hair growth study

Hairs from 2 cm^2 area at the dorsal portion of all the mice were shaved using electric shavers and applied with marketed hair remover to completely remove hair. After 9^{th} day of depilation all hairs came at anagen VI stage. Cyclophosphamide (50mg/kg i.p.) was injected on the 9^{th} day to induce hair loss. Daily 0.2 ml of test samples, vehicle and standard samples were applied topically on back skin for 20 days. For the next few days mice were observed for feed and water intake. On 20^{th} day of study mice from each group were selected randomly for skin biopsy from balding site and samples were kept in phosphate-buffered formalin. Vertical sections of skin were cut parallel to the direction of hair growth and stained with haematoxylin and eosin for histology. A/T ratio and hair follicular density was determined with the help of ocular micrometre (Patel et al., 2014 and Sabharwal et al., 2009).

2.7 Statistical analysis

The results were expressed as mean ± SEM. The data were statistically evaluated by one-way ANOVA, followed by Dunnet's t-test for comparison of test groups with control. Values of $p < 0.05$, $p<0.001$ and $p < 0.001$ were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Pharmacognostical study

3.1.1 Macroscopy

Leaves were green in obovate, ovate round in shape, obtuse apex, symmetrical base, glabrous surface and 2.1-2.4 by 1.3-2.0 cm. The colour of fresh leaf at upper surface was dark green and greyish green at lower surface while dried leaves were yellow from upper surface and light yellow from lower surface (Fig. 1).

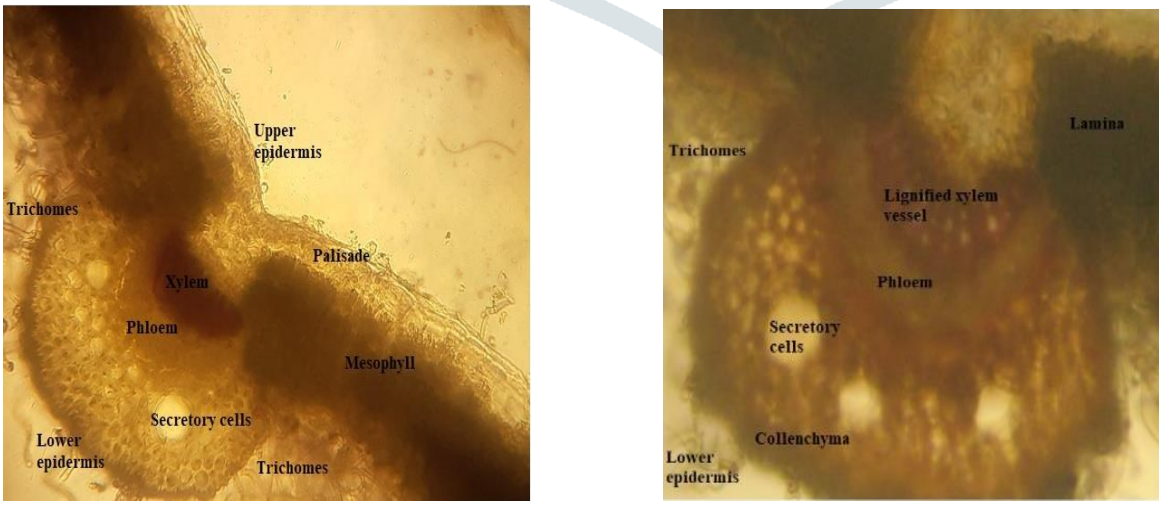
Upper surface Lower surface

Fig. 1.: Colour of *Ziziphus nummularia* (Burm. f.) Wight &Arn. fresh leaf at upper and lower surface

3.1.2 Microscopy

Transverse section of *Z. nummularia* leaf

Transverse section passing through midrib was slightly curved on the upper surface and strongly bulging at the lower surface as showed in Fig. 2 (A). It showed single layered upper epidermis and lower epidermis. Palisade cells were seen below upper epidermis in lamina portion. Midrib showed presence of vascular bundles having lignified xylem vessles. Closed collateral type of vascular bundle was present. Lower portion of the midrib was occupied by collenchymatous cells. Trichomes were seen at upper and lower epidermis of leaf Fig. 2 (B). Leaf constants such as stomatal number, stomatal index, vein-islet number and veinlet terminations number are shown in Table 1.



(A) **(B)**

Fig. 2 (A) T.S. of *Z. nummularia* leaf (B) enlarged view

Powder microscopy

Powder microscopy of leaf showed numerous trichomes, calcium oxalate crystals,anomocytic type of stomata, vessels, lignified and non-lignified fibers as shown in Fig. 3.

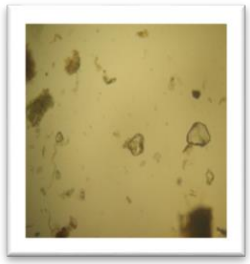
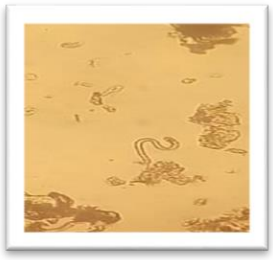
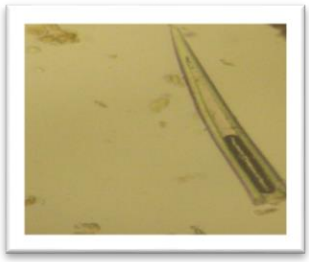
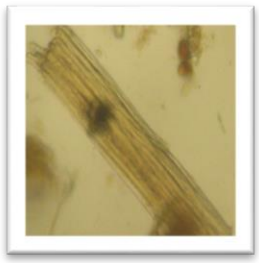
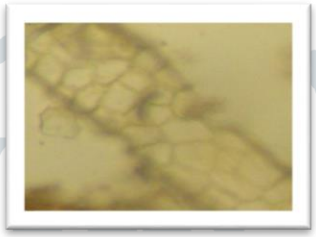
		
Calcium oxalate crystals	Fibers	Trichomes
		
Vessels	Lignified fibers	Parenchyma cell

Fig. 3 Powder microscopy of *Z. nummularia* leaf powder

Table 1. Leaf constants of *Z. nummularia* leaves

Sr. no.	Parameters	values in 1mm^2 area
1	Stomatal number (upper epidermis)	1.3
2	Stomatal index (upper epidermis)	9.1
3	Veinlet	12-15
5	Vein islet termination	2-4
7	Palisade ratio	4-5 (per cell)

3.1.3 Physicochemical analysis

The results of various parameters are given in the Table 2. These physicochemical parameters will be functional for identification of *Z. nummularia* even in its powdered form.

Table 2. Physicochemical parameters of *Z. nummularia* leaf

S. no.	Physicochemical parameters	Values
1	**Ash values (% w/w)**	
	Total ash	7.52±0.60
	Acid insoluble ash	1.5±0.23
	Water insoluble ash	3.02±0.12
	Sulphated ash	0.712±0.013
2	**Extractive values**	
	Ethanol extractive value (% w/w)	
	Hot extraction method	9.23 ±0.1
	Cold maceration method	4.28 ±0.2
	Aqueous extractive value (% w/w)	
	Hot extraction method	12.4 ±0.9
	Cold maceration method	5.48± 0.4 1
3	Foreign organic matter	0.7%
4	Loss on drying	8.2±1.25 % w/w
5	Swelling index	42± 0.121 % v/v
6	Foaming index	1000
7	Haemolytic activity	No haemolysis
8	Crude fibre content	42%
9	Aflatoxin presence	Nil
10	**Microbial count determination**	
	Total aerobic count	300 CFU/g
	Total fungal count	25 CFU/g
11	**Pathogen analysis**	
	Escherichia coli	Absent
	Salmonella typhi	Absent
	Pseudomonas aeruginosa	Absent
	Staphylococcus aureus	Absent
	Clostridia	Absent
	Shiegella	Absent

Values in % w/w and %v/v are expressed as mean± SEM; n=3

3.1.4 Fluorescence analysis

The leaf powder was treated with different chemical reagents and results are reported in Table 3.

Table 3 Fluorescence analysis of *Z. nummularia* leaves powder

Powdered Drug +reagent	Visible/Day light	UV 254 nm (short)	UV 365 nm (long)
Powder as such	Light green	Black	Black
Powder + 5% NaOH	Brown	Black	Dark brown
Powder + acetic acid	Green	Black	Black
Powder + distilled water	Green	Black	Brown
Powder +conc. H_2SO_4	Brown	Black	Black
Powder +dilute H_2SO_4	Green	Black	Dark brown
Powder + conc. HCl	Dark green	Black	Black

Powder+ dil. HCl	Green	Dark brown	Dark brown
Powder + ammonia	Green	Black	Black
Powder +ethyl acetate	Green	Dark green	Black
Powder +chloroform	Light green	Black	Black
Powder + ethanol	Brown	Black	Dark brown

3.1.5 Elemental analysis

The powdered leaf showed elemental contents Pb, As, Co, Cu, Cd, Hg, Mg, Fe, Mn, Ca and K with in limits. The results are shown in the Table 4.

Table 4: Heavy metal concentration in *Z. nummularia* leaf powder

S. no.	Elements	*Z. nummularia*
1	Lead	0.631
2	Arsenic	0.000
3	Copper	0.000
4	Cadmium	0.013
5	Mercury	0.709
6	Magnesium	0.025
7	Iron	1.510
8	Manganese	0.043
9	Calcium	4.116
10	Potassium	0.547
11	Cobalt	0.097

3.1.6 Quantitative analysis

The quantitative analysis was determined for phenolics, flavonoids, saponins andalkaloids contents in selected plants extracts.

Determination of total phenolic content

The gallic acid solutions of different concentration (20-100 µg/ml) confirmed to Beer's Law at 760 nm with regression co-efficient (R^2) = 0.998. The equation of standard curve is y = 0.0022 x + 0.0872 as shown in Fig. 4. Total phenolic content is shown in Table 5.

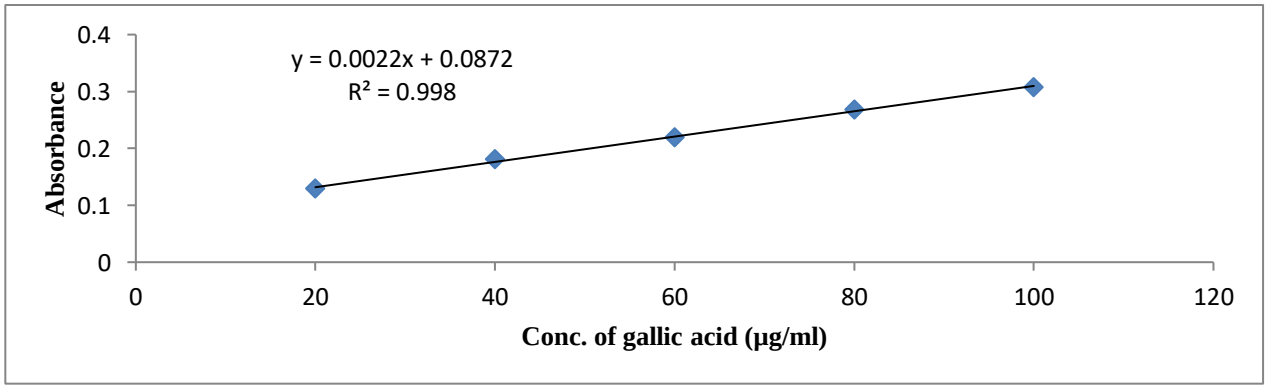


Fig. 4 Standard calibration curve for total phenolic content

Determination of total flavonoid content

The solutions of quercetin (20-100 µg/ml) confirmed to Beer's Law at 510 nm with a regression co-efficient (R^2) = 0.998. The equation of standard curve is y = .001 x+ 0.06 as shown in Fig. 5 and total flavonoid content is shown in Table 5.

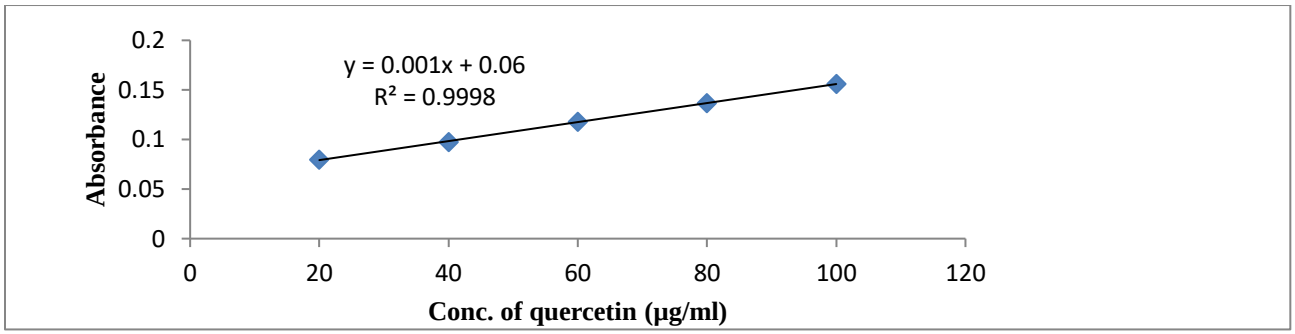


Fig. 5: Standard calibration curve for total flavonoid content

Determination of total tannin content

The solutions of gallic acid (20-100µg/ml) confirmed to Beer's Law at 725 nm with regression co-efficient (R^2) = 0.9981. The equation of standard curve is y = 0.0008x + 0.0143 as shown in Fig. 6 and total tannin content is shown in Table 5.

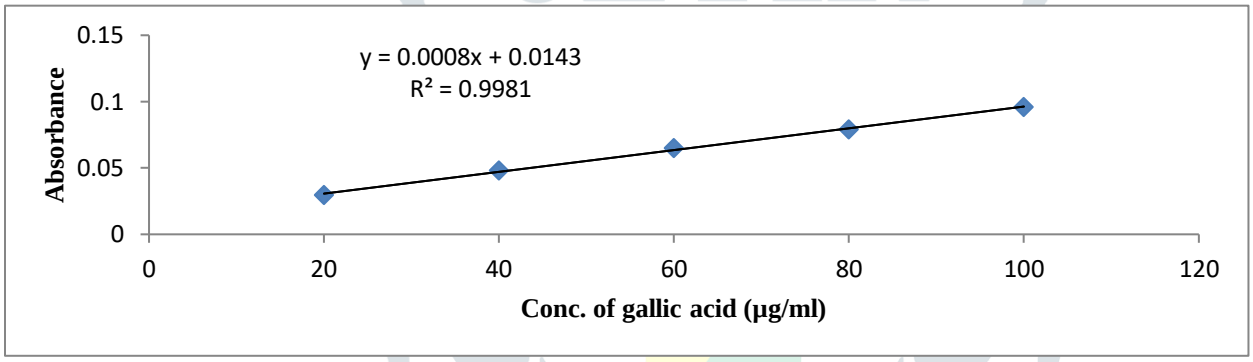


Fig. 6: Standard calibration curve for total tannin content

Determination of saponin content

Saponin content was expressed as mg/g of dried fruit powder and shown in Table 5.

Determination of alkaloid content

The total alkaloid content was examined in extracts and expressed in terms of atropine equivalent as mg of AE/g of extract. The standard curve equation: y = 0.0068x – 0.0562, R^2 = 0.9986 as shown in Fig.7 and the alkaloid content is shown in Table 5.

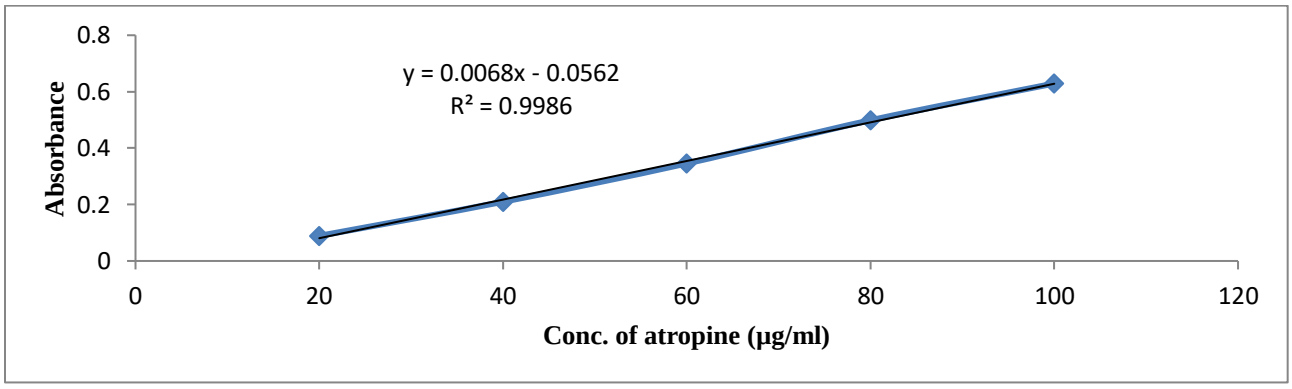


Fig. 7: Standard calibration curve for total alkaloid content

Table 5: Total phenol, total flavonoid, total tannin, saponin and total alkaloid content of *Z. nummularia* leaves

Phytocontent	Value
Total phenol (mg of gallic acid equivalents/g of dry weight)	43.85±1.24
Total flavonoid (mg of quercetin equivalents/g of dry weight)	63.067±0.62
Total tannin (mg of gallic acid equivalents/g of dry weight)	44.375±1.61
Saponin content (mg/g of the dried drug powder)	49±1.73
Total alkaloid (mg of AE/g of dry weight)	36.56±0.13

Values are expressed as mean ±SEM (n=3)

3.2 *In-vitro* antioxidant study

In-vitro antioxidant activity was performed by DPPH free radical scavenging activity, reducing power, H_2O_2 scavenging activity and metal chelation methods. Results of all the models reflect that *Z. nummularia* leaves hydroalcoholic extract showed better antioxidant activity than n-hexane extract (Figs. 8, 9, 10 and 11). Table 6 depicting IC_{50} value. Hydroalcoholic extract has lower IC_{50} value as comparison to n-hexane extract so indicating better antioxidant activity.

Table 6. IC_{50} of *Z. nummularia* extracts

S. no.	Antioxidant method	IC_{50}(µg/ml)		
		Standard	ZE	ZH
1	DPPH free radical scavenging activity	27.71±1.097	41.64±2.34	100.89±5.64
2	Hydrogen peroxide activity	250.9±22.03	395.24±13.821	1162.24±39.76
3	Metal chelation method	21.1±0.836	16.86±0.5776	106.86±0.8581

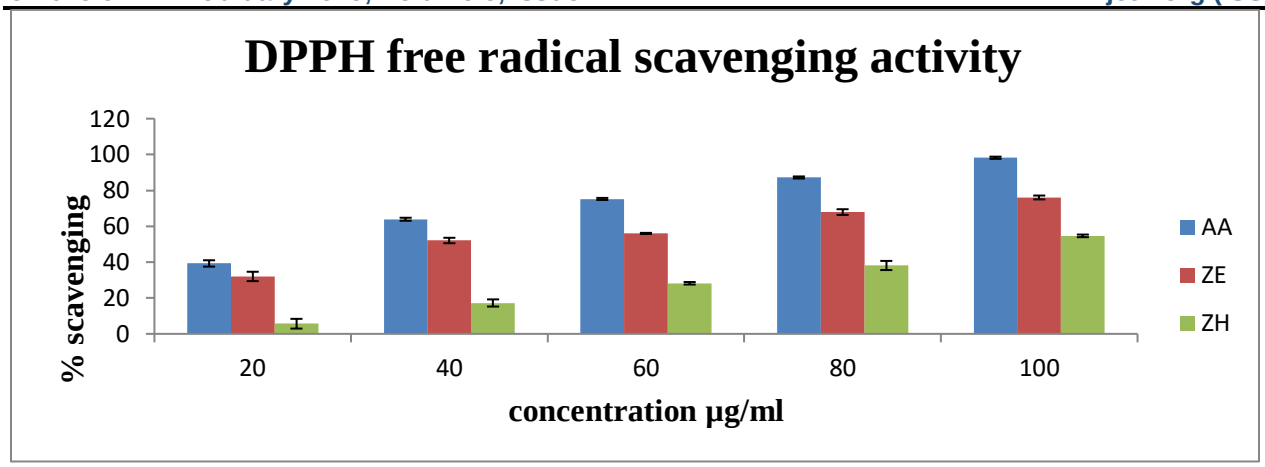


Fig. 8 DPPH free radical scavenging activity of ascorbic acid (AA) as standard, hydroalcoholic (ZE) and n-hexane (ZH) extracts of *Z. nummularia* leaves. Each value represents as mean ± SEM (n=3)

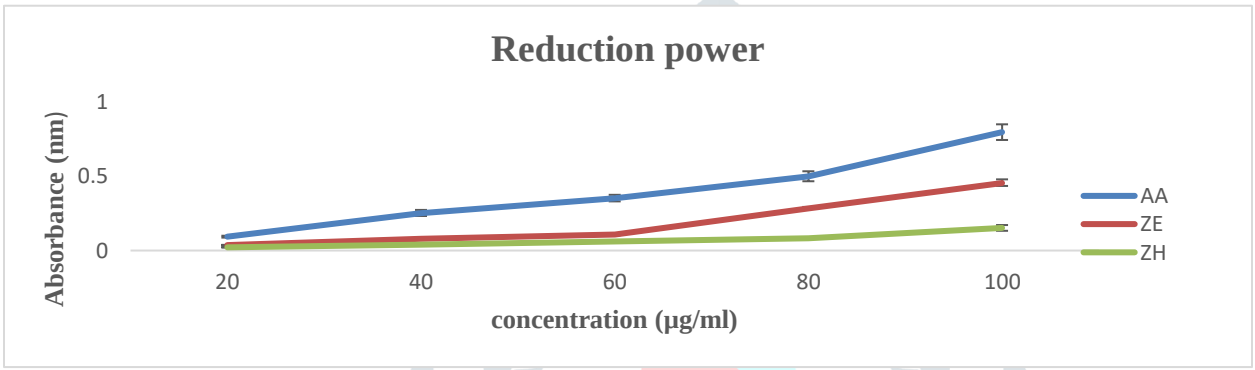


Fig. 9. Reducing power activity of ascorbic acid (AA) as standard, hydroalcoholic (ZE) and n-hexane (ZH) extracts of *Z. nummularia* leaves. Each value represents a mean ± SEM (n=3)

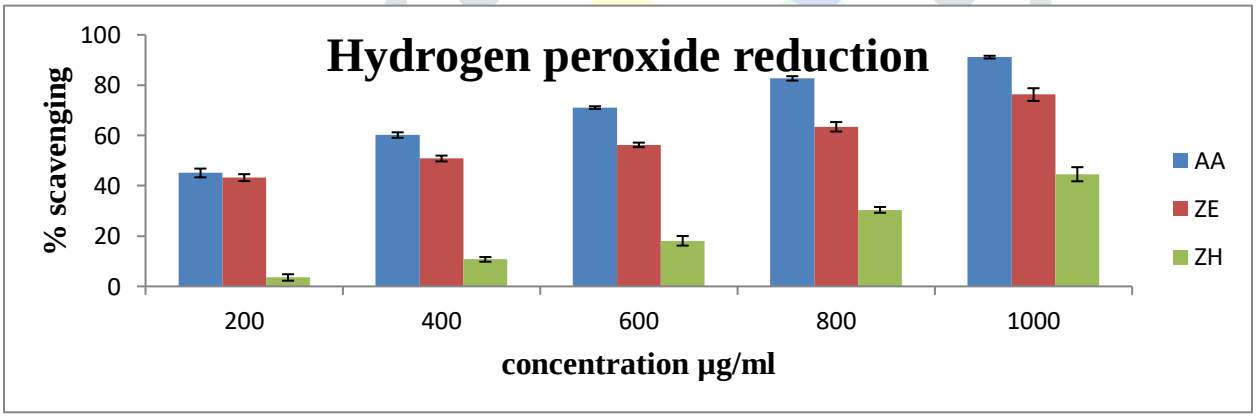


Fig. 10. Hydrogen peroxide scavenging activity of ascorbic acid (AA) as standard, hydroalcoholic (ZE) and n-hexane (ZH) extracts of *Z. nummularia*leaves. Each value represents a mean ± SEM (n=3)

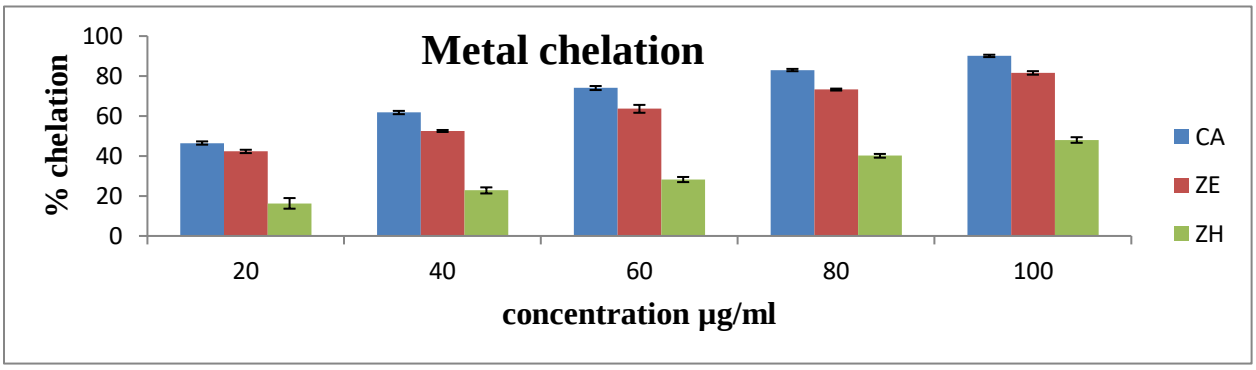


Fig. 11. Metal chelating activity of citric acid (CA) as standard hydroalcoholic (ZE) and n-hexane (ZH) extracts of *Z.*

***nummularia*leaves. Each value represents a mean ± SEM (n=3)**

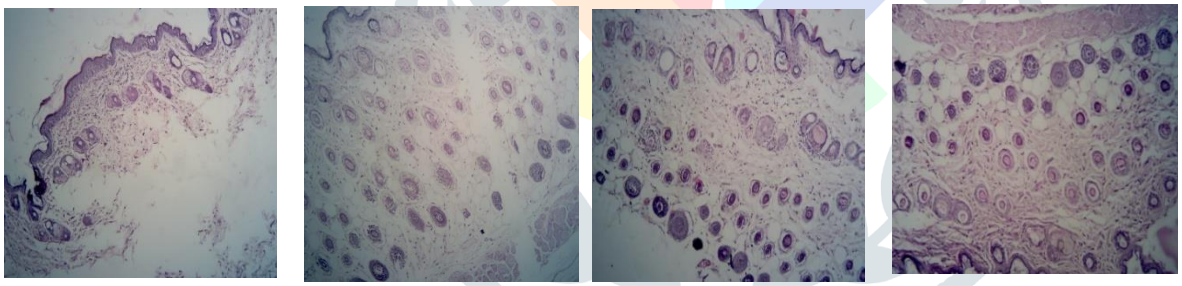
3.3 Hair growth study

3.3.1 Morphological observation

Hair growth at shaved sites were mostly covered by day 14. In animal group 1 in the first 9 days of study, they had normal feeding pattern, but after administration of cyclophosphamide during from day 15th feeding rate was dropped down. The grown hair at shaving sites were very weak. In group 2 and 4 there were no significant variations in feeding pattern. Hair regrowth at depilated sites was slow up to 13 days but after that up to, 19th day of study full regrowth was observed. In animals of group 3 there were no significant variations in feeding pattern. Hair growth on day 14th was less comparatively to standard was but more than control. Hair growth at depilated site was not visible in all animals on 19th day of study.

3.2.2 Histpathological observation

Animals of group 2 revealed focal areas of normal hair follicles and numerous maturing hair follicles while in group 1 animal skin showed disruption of melanin granules and epidermis, irregular diameter of hair bulbs and distortion of hair follicles. In animal group 3 their skin showed thickened and regular epidermis, few distorted hair follicles along with normal hair follicle and irregular diameter as compared to standard drug treatment but more than control. Group 4 animal treated skin showed normal epidermis, a greater number of hair follicles, wide open hair canal, presence of both anagen and telogen cyclic phase, uniform melanin granules, less distorted hair follicles were present shown in Fig. -12



Group 1 Group 2 Group 3 Group 4

Fig. 12 Histopathological changes on mice skin, photomicrographs (10X) of H &E stained skin sections. Group 1- control group, vehicle treated showing distorted and lesser no. of hair follicles; Group 2- Standard group, minoxidil (5% w/v in ethanol, topical) melanin containing mature hair follicles; Group 3- test group n-hexane extract treated (30% w/v, topical) irregular diameter and lesser anagen hair follicles; Group 4- test group hydroalcoholic extract (30% w/v, topical) treated group showing more no. of anagen hair follicles with regular diameter

3.3.3 Hair follicular density

The histological study (Fig. 13) showed that group 1 animal showed hair follicular density was very less. Hair follicular density was observed most significant ($p<0.001$) in group 2 and 4 as compare to group 1. Group 3 showed significant ($p<0.01$) hair follicular density as compare to group 1.

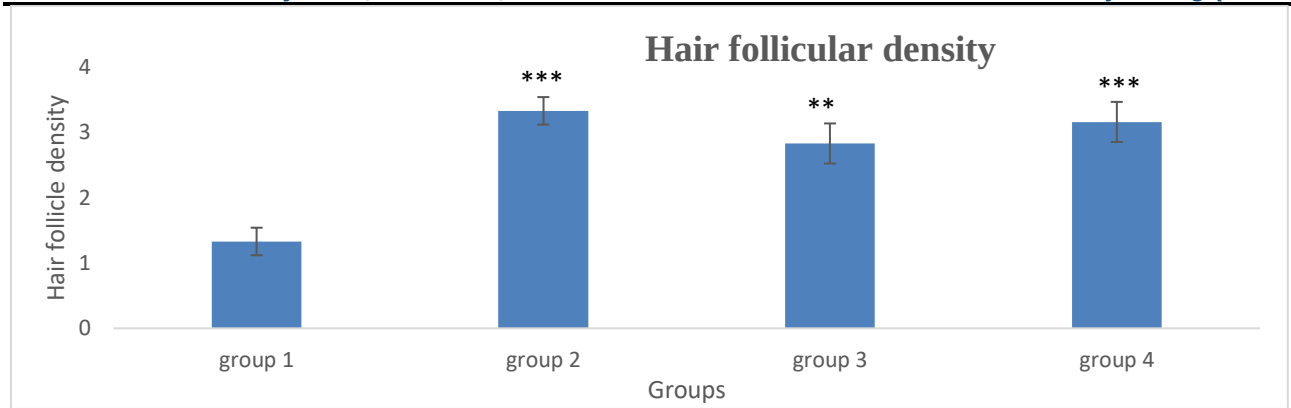


Fig 13 Hair follicular density where, Group 1– vehicle, Group 2 – standard (minoxidil), Group 3- n-hexane of *Z. nummularia*, Group 4 – hydroalcoholic extract of *Z. nummularia*, treated animals. Values are expressed as Mean ± SEM, in each group. Data was analyzed by one way ANOVA followed by Dunnett's t-test, *p<0.05, **p<0.01, ***p<0.001, significance versus group 1 (vehicle)

3.3.4 Anagen:telogen ratio

The histopathological studies showed that minoxidil treated animals hair follicles were maximum in anagen phase, followed by hydroalcoholic extract treated animals. Meanwhile, n-hexane extract treatment showed lesser number of hair follicles in anagen phase more in telogen phase, vehicle treated group's hair follicle were maximum in telogen phase as shown in Table 7.

Table 7. Anagen/Telogen (A/T) ratio in skin sections of different groups of animals

Group	A/T ratio
Group 1	0.416 ±0.1667
Group 2	3.667±0.333***
Group 3	1.767±0.233
Group 4	3.333± 0.333***

Groups 1– Control, Group 2 – Standard, Group 3- n-hexane treated, Group 4 – hydroalcoholic extract of *B. vulgaris* treated animals. Values are expressed as Mean ± SEM, in each group (n=6). Data was analyzed by one-way ANOVA followed by Dunnett's t-test, with significant *p<0.05, **p<0.01, ***p<0.001value compared to control

Cyclophosphamide-induced alopecia (CIA) results from induction of follicle dystrophy and the premature induction of follicle regression (catagen) in growing (anagen) hair follicles. Proteins encoded by p53 target genes (Fas, Bax, insulin growth factor receptor type I (IGF-RI) are up-regulated after cyclophosphamide administration (Lindner et al., 1997). Oxygen free radical scavenger protects against cyclophosphamide induced alopecia in animals (Conklin et al., 2004).

GC-MS analysis showed presence of phytosterols like stigmasterol, beta- sitosterol, vitamin- E and phytol (Sonia and Singh, 2018). Phytosterols, Vitamin E and phytol are having a good free radical scavenging activity (Yoshida and Niki, 2003; Camila et al., 2013). Phytochemicals like flavonoids, phenolic acids, and triterpenoids plays a major role in preventing cyclophosphamide induced alopecia due to their antioxidant activity and probably by down regulating p53 target genes expressed in the hair follicle. Hydroalcoholic extract showed better antioxidant activity that is why this extract showed better anti-alopecic activity than n- hexane leaves extract. From histological study it is clear as hydroalcoholic extracts treatment showed uniform melanin

granules, less distorted hair follicles, regular diameter of hair follicles, hair shaft and fragments and more anagen hair follicles comparatively to n-hexane extract.

As *Z. nummularia* leaves extracts possess antioxidant activity that is due to presence of flavoinoids, phenolic acids and tannins, so they can prevent reactive oxygen species from damaging hair follicles. Free radical scavenging properties of test extracts help to prevent hair loss by preventing accumulation of p53, because p53 causes growth arrest. n- hexane and hydroalcoholic extracts of *Z. nummularia* leaves showed the ability to prevent hair follicle damage by cyclophosphamide. Thus *Z. nummularia* have demonstrated its ability to cure alopecia and damage to skin structure caused by cyclophosphamide.

4. CONCLUSION

Pharmacognostical studies can be used as a diagnostic tool for the standardization of *Z. nummularia* L. leaves to facilitate genuity, quality control, identification, preparation of a monograph and to minimize the adulteration. Different phytoconstituents present in n-hexane and hydroalcoholic leaf extracts of *Z. nummularia* responsible for *in-vitro* antioxidant potential. Hydroalcoholic extract showed better *in-vitro* antioxidant and anti- alopecic activity than n-hexane extract. Thus *Z. nummularia* plant extracts can be used as antioxidant and for the treatment of cyclophosphamide induced alopecia. However, additional study is required to further explore the downstream signaling pathways involved in the cyclophosphamide induced alopecia in animals.

Conflict of interest: There is no conflict of interest among authors.

Acknowledgement

Authors are thankful to Guru Jambheshwar University of Science & Technology, Hisar for laboratory facility and University Research Scholarship provided to support this research work financially.

References

1. Rathore M. Nutrient content of important fruit trees from arid zone of Rajasthan. Journal of Horticulture and Forestry 2009; 1(7): 103-108.
2. Kumar Sushil, Vipin Kumar Garg, Nitin Kumar, Pramod Kumar Sharma, Sudhir Chaudhary and Anshu Upadhyay, 2011. Pharmacognostical studies on the leaves of *Ziziphus nummularia* (Burm. F.). European Journal of Experimental Biology, 2011, 1 (2):77-83.
3. Macquart M.G., Viens, P., Bouscary, M.-L., et al., 1997. Discordance between physicians' estimations and breast cancer patients' self-assessment of side-effects of chemotherapy: an issue for quality of care. Br J Cancer. 76, 1640-1645.
4. Valdimir, A., Botchkarev, 2003. Molecular Mechanisms of Chemotherapy-Induced Hair Loss. Jid symposium proceeding. 8 (1) 72-75.
5. Peus, D., Pittelkow, M.R., 1996. Growth factors in hair organ development and the hair growth cycle. Dermatol Clin. 14(4), 559–572. [PubMed: 9238316]

6. Patel S., Sharma V., Chauhan N.S., Dixit V.K. A study on the extracts of *Cuscuta reflexa* Roxb. in treatment of cyclophosphamide induced alopecia. DARU Journal of Pharmaceutical Sciences 2014 22:7.
7. Batchelor, D., 2001. Hair and cancer chemotherapy: consequences and nursing care—a literature study. European Journal of Cancer Care. 10 (3), 147-163.
8. Pandya DJ, Desai TR, Nadpara NP, Mehta HA, Modi AM (2010). Pharmacognostic study and establishment of quality parameters of leaves of *Bombax insigne* Linn., Int J Pharmacogn Phytochem Res. 2(3): 1-5.
9. Khandelwal KR (2008). Practical pharmacognosy. 19th ed. Pune: Nirali publication; p. 149-164.
10. Mukherjee PK: Quality control of herbal drugs, an approach to evaluation of botanicals. Business Horizones Pharmaceutical Publishers, Kolkata, India, First edition 2002; 131-148, 183-422.
11. Anonymous**.** Quality Control Methods for Medicinal Plants Material, World Health Organization, Geneva. AITBS publishers and distributors (Regd.), Delhi, India, 2004; 6-64.
12. WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues, WHO press publishes, Spain, 2007.
13. Singh Sumitra and Sonia (2018) Pharmacognostical evaluation and phytochemical analysis of *Delonix regia* Rafin. stem bark, *IJPSR, Vol. 9(5): 1908-1912.*
14. Pratt RT and Chase ER. Fluorescence powder vegetable drugs in particular to development system of identification. J Am Pharm Assoc 1949**;** 38**:** 324-331**.**
15. Indian Pharmacopoeia. Ghaziabad, India: The Indian Pharmacopoeia Commission, Government of India, 2007, 1.
16. AOAC. AOAC official methods of analysis. 15th ed. Association of Official Analytical Chemists, Arlington,Virginia. 1990, 84-85.
17. Singleton VL, Orthofer R, Lamuela-Raventos RM (1999): Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299, 152-178.
18. Singh R, Verma PK, Singh G (2012) Total phenolic, flavonoids and tannin contents in different extracts of *Artemisia absinthium*, J Intercult Ethnopharmacol. 1 (2): 101-104.
19. Theresa Ibibia Edewr, Stephen olugbemiga owa, adeola opeyemi ologanand franklin akinfemi, 2016. Quantitative determination of the saponin content and GC-MS study of the medicinal plant Cassytha filiformis (linn.)leavesjournal of coastal life medicine. ,4(2); 154-156.
20. Manjunath A, Mahadev BG, Shradda UN (2012). Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer, Anc Sci Life. 31(4):198–201.

21. Shamsa F.Monsef H.Ghamooshi R.Verdian-rizi M. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. ThaiJournal of Pharmaceutical Sciences. 2008; 32: 17-20.
22. Arumugam, K. and Venugopal, S., 2016. Phytochemical studies, antioxidant activities and identification of active compounds using GC-MS of *Dryopteris cochleata* leaves. Arab. J. Chem. 9, S1435- S1442.
23. Roduan M.R., Hamid R.A. and Cheah Y.K. Cytotoxicity, antitumor-promoting and antioxidant activities of Annona muricata in vitro. Journal of Herbal Medicine. 2019, vol. 15, 1-6.
24. Sroka, Z., Cisowski W., 2003. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food and Chemical Toxicology. 41, 753–758.
25. Soler-Rivas C, Espin JC, Wichers HJ, 2000. An easy and fast test to compare total free radical scavenger capacity of foodstuffs; *Phytochem. Anal*., 11: 330-338.
26. Sanchez-Moreno, C.; Larrauri, J.A.; Saura-Calixto, F. A procedure to measure the antiradical efficiency of polyphenols. J. Sci. Food Chem. 1998, 76, 270–276.
27. Rivero-Cruz JF, Granados-Pineda J, Pedraza-Chaverri J, Pérez-Rojas JM, Kumar-Passari A, Diaz-Ruiz G, Rivero-Cruz BE. Phytochemical constituents, antioxidant, cytotoxic, and antimicrobial activities of the ethanolic extract of Mexican brown propolis. Antioxidants. 2020 Jan;9(1):70.
28. Sabarwal N., Varghese D., Barik R., Khandelwal A., Jain A., Jain S., 2009. Development and Evaluation of Polyherbal Formulations for Hair Growth Activity. Phcog. Net. 1(2), 165-170.
29. Lindner, G., Botchkarev, V.A., Botchkareva, N.V., Ling, G., Van, V.C, Paus, R., 1997. Analysis of apoptosis during hair follicle regression (catagen). Am J Pathol. 151, 1601- 1617.
30. Conklin, K. A., 2004. Chemotherapy-associated oxidative stress: impact on chemotherapeutic effectiveness. Int Cancer Therapies 3 (4): 294-300.
31. Sonia and Singh S; GC-MS analysis of Phytoconstituents of *Ziziphus nummularia* (Burm. f.) Wight &Arn. Leaves Extracts Using GC-MS Spectroscopy. Research and reviews: A Journal of life sciences. 2019, 109-117.
32. Yoshida, Y., Niki, E., 2003. Antioxidant effects of phytosterol and its components. J Nutr Sci Vitaminol (Tokyo). 49(4), 277-80.
33. Camila, C. et al., 2013. Antinociceptive and Antioxidant Activities of Phytol In Vivo and *In Vitro* Models. Neuroscience Journal. 1-9.