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Contents

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Research Article

Diameter at breast height-crown width prediction models for *Parkia biglobosa* (Jacq) R. Br. ex G. Don.

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ABSTRACT

This study was carried out in Kano state, Nigeria with the objective of predicting crown width from diameter at breast height and the planting spacing of *Parkia biglobosa* (Jacq) species. This is an economic plant with multipurpose usage. This species is exposes to degradation, overexploitation and have a regeneration problems. There is lack of information on inventory for *P. biglobosa* (Jacq) in term of plantation establishment and management. Data from the economic tree species were collected from nine plots (sizes 100 x 100 meters each), randomly laid down. Data on trees diameter at breast height (dbh) and crown diameter were collected from each plot within the study area. The data collected were tested on five crown and stem diameter relationship models. The simple linear model was best fitted with R^2 values of 0.69 to 0.85. The results shows that a dominant free-growing tree species with diameter 50.66 cm would require 0.0066 hectare of growing space with a stocking of the stand in terms of total occupancy by tree crowns of 151 trees per hectare. For optimum planting distance, fast growth and high production/yield, *P. biglobosa* would require a planting spacing of 4 x 4 meters (for NTFPs purpose) and 8 x 8 meters (for timber purpose). Thus, the baseline information from this study could serve as a guide for optimum planting distances and tree density in large scale plantations for countries with similar species and forest types.

Keywords: Crown width; Bole diameter; Prediction; Models; Economic.

1. INTRODUCTION

Parkia biglobosa (Jacq) is found in a wide range of environments in Africa including Nigeria. It is a dicotyledonous angiosperm which is categorized under vascular plants in the family Fabaceae - Mimosoideae [1]. It is a deciduous perennial plant that grows up to between 7 and 20 meters height in some cases up to 30 meters [2]. The species is fire-resistant, characterized by a thick dark grey-brown bark. The pods of the tree, commonly referred to as locust beans, are pink in the beginning and turn dark brown when fully mature. They are 30-40 cm long on average, with some reaching lengths of about 45 cm in length. Each pod can contain up to 30 seeds.

P. biglobosa species has several uses, including fodder, food, medicine, green manure, fuel wood, timber and economic purposes [3]. The cultivation of this tree can be seen as an

important economic activity for many developing countries [4]. The species is commonly known as "African locust bean". The plant is a very important, multi-purpose tree, it is commonly gathered from the wild for local use as food, medicine and to provide a wide range of commodities. Indigenous healers in Africa use different parts of the locust bean tree for health benefits. In a survey conducted on healers in Togo, *P. biglobosa* was one of the highest cited plants used for treating hypertension [5]. In a survey conducted in Guinea relating to their use of anti-malarial plants, *P. biglobosa* was cited among those most often successfully used [6].

The prediction of crown width for sustainable management of *P. biglobosa* species require reliable model to determine stand density and stocking of the tree species. Crown width is relating to growth space. The knowledge on crown width has been employed in growth modeling studies, based not only on its measure as the ground area or resources available to individual trees, but also as an indication of competition [7, 8]. Growth space refers to the adequate resources required within a given unit of land for the germination, emergence, growth and development of tree species. An inadequacy of any of these resources needed by individual trees in a stand may limit the growing space and hence affect tree growth [7].

The population of *P. biglobosa* species is rapidly declining, and there are no conservation efforts to prevent it from extinction in most of the developing countries [9]. The continuous removal of this tree species (forest ecosystem) could affect mankind directly or indirectly because of the species economical value. The objective of this study was to develop model to predictcrown width for *P. biglobosa* (Jacq) species for proper management of the species for timber and non-timber forest products purposes. Thus, present study on crown width, its present structure, growth and yield would be of great importance to mankind and forests managers for optimum planting distance and sustainability.

2. MATERIAL AND METHODS

2.1. Study area

This study was carried out on *P. biglobosa* in Kano-Nigeria. It lays between latitude 12^{o} 25′ to 12^{o} 40′ N and longitude 8^{o} 35′ N to 8^{o} 45′ E. Kano state is located in the northwest geo-political zone (Sudan savanna vegetation) of Nigeria. Hausa and Fulani, who are predominantly Muslims, inhabit Kano State. The State is having a population of about 9, 383, 682 [10]. It has 44 local governments, with an area of 20,479.6 square kilometer. The annual rainfall is between 75 mm to 1000 mm in the northern part and increases to 1,120 mm in the southern part of the state. The rainy season lasts within the months of May to September. The topography is of high-level for a few hills in the southern part.

2.2. Measurement procedures

P. biglobosa was selected based on its economic values to communities. Simple random design was used to locate nine sample plots sized 100 x 100 m each within the study area. Complete enumeration of trees greater than 10 cm diameter at breast height (dbh) was carriedout and the measurements of the parameters of interest were taken. The data collected on every sampled tree includes: crown width by using 30-meter measuring tape and stem diameter at breast height (dbh). Diameter of the sampled trees was determined with the use of diameter tape on winding the tape around the tree at point of measurement (1.3 m) above the ground on the uphill side of the tree [9].

Crown-width measurement was based on the assumption that the vertical projection of a tree crown is circular; four radii were measured (using 30-meter measuring tape) and in the direction forming equal angles [7, 8]. Along each radius of the tree crown, the diameter tape was held horizontally and extended until each person was vertically under the tip of the longest branch on both sides. A 3-meter ranging pole was used to align vertically to the edge of the crown [9]. The diameter tape was turned by 90^{o} and measurements were carried out repeatedly along the thinnest part of the tree crown and recorded [7]. Average crown width (Cw) was calculated by summed up the four radii and divided by 2.

2.3. Data analyses

2.3.1. Basal area estimation

The diameter at breast height (dbh) was used to compute basal area using the formula:

$B.A = \pi D^2 / 4$ Equation (2) [7]

Where: BA = Basal area (hectare); D = Diameter at breast height (m) and $\pi = 3.142$.

2.3.2. Crown-width

The data collected from the field were fitted to the following model forms for predicting crown width suggested by different authors with the aim of choosing the model form that showed the best ability to stabilize the variance in the data:

- $Cd = b_o + b_1\, dbh + ei$ Equation (3a) [11]
- $Cd = b_o + b_1 dbh^2 + ei$ Equation (3b) [7]
- $Cd = b_o + b_1 dbh + b_2 \ln dbh + ei$ Equation (3c) [9]
- $Cd = b_o + b_1 \ln dbh + ei$ Equation (3d) [8]
- $\ln Cd = b_o + b_1 dbh^2 + b_2 \ln dbh^2 + ei$ Equation (3e) [7]

Where: Cd = crown diameter; dbh = diameter at breast height; b_0 = intercept, b_1 and b_2= regression coefficient.

2.3.3. Crown area

Using the calculated crown diameter (Cd), the crown area (A) was predicted and expressed in hectare basis (conversion of crow diameter in meters to area in hectares):

$A = (\pi\, Cd^2/ 4) / 10{,}000$ Equation (4) [7]

Where: A = growing space/area; Cd = crown diameter; $\pi = 3.142$

$N = 1/A$ i.e. $[1/(Cd^2/ 4)/10{,}000]$ Equation (5) [9, 12, 13]

Where: N = stock; A = growing space.

2.3.4. Stand density/ Basal area per hectare

$S.D = \pi D^2 / 40{,}000$ Equation (6) [8]

Where: SD= stand density; D= diameter; $\pi = 3.142$.

2.4. Criteria for models selection and ranking

The models were fitted and assessed. The assessment was based on all the following criteria: the significance of regression equation (F-ratio), coefficient of determination (R^2), root mean square error (RMSE) and normal probability plots of residuals [9, 14]. The best model was selected based on a ranking procedure using the above mentioned criteria. The model with the highest F-ratio or R^2 was assigned a rank of one while model with the lowest F-ratio or R^2 was assigned a rank of five.The model with best fitted residual or normal probability plots and lowest RMSE was assigned a rank of one. Then the rank for R^2, RMSE, F-ratio, residual and normal probability plots were combined, and were then summed to determine the most appropriate model [15, 16]. T-test and ANOVA were used to test for significant difference at $p \leq 0.05$ level of significant. Twenty five percent of data from the tree species were used (independent of those used to develop the model) for the purpose of validation.

3. RESULTS AND DISCUSSION

Figures 1a and 1b show the class distribution of bole diameter and crown diameter. This study showed that there was more concentration of stem diameter at the lower diameter distribution (15.00-40.00 cm) than in the upper diameter distribution class (41.00-50.1 cm). This may be as a result of high exploitation or mortality on the tree species in the area. Table 1 presented the model parameters on *P. biglobosa* species from the study area. Coefficients of determination (R^2) ranged from 0.69 (coefficient correlation = 69%) to 0.85 (coefficient correlation = 85%). RMSE was used to compare untransformed models while *F.I* was used to compare the transformed models. It ranged from 0.20 to 0.80 and F-ratio ranged from 101.0 to 1116.5 (Table 1).

The result on the crown diameter class can be used as an important visual indicator of tree and forest trend (healthy or unhealthy) in the study area. Trees with full and healthy crowns are generally associated with higher growth rates as a result of an increased rate for photosynthesis. These results described the current status and condition of *P. biglobosa* species in the area (Figures 1a and 1b). The figures revealed the crown condition of the study area which was healthy and free from high competition. This may be as a results of low population (declining in population due to deforestation without reforestation) of the tree species (open grown trees) or the soil condition of the area. The result is in accord with

Lawrence [17], who reported that crown degradation is typically the result of past and present stressors such as insects, diseases, weather events, drought and competition or other stand conditions and when severe enough, may result in tree mortality.

Table 1. Model parameters on *P. biglobosa* species in the study area.

Model No.	Model type and model coefficients	R^2	RMSE	F-ratio	FI
[3a]	Cd = 2.244 +0.137 dbh	0.84	0.68	1116.5	-
[3b]	Cd = 3.985+ 0.021 dbh^2	0.80	0.80	795.06	-
[3c]	Cd =0.705 + 0.88 dbh + 1.56lndbh	0.80	0.78	658.66	-
[3d]	Cd = - 4.913+ 4.422 lndbh + ei.	0.85	0.75	101.0	-
[3e]	LnCd = 0.212 – 0.016 dbh^2 + 1.56 $Lndbh^2$	0.69	0.20	348.77	0.078

dbh = Diameter at breast height(cm); Cd = Coefficient of Determination; RMSE = residual mean square error; FI = furnival index.

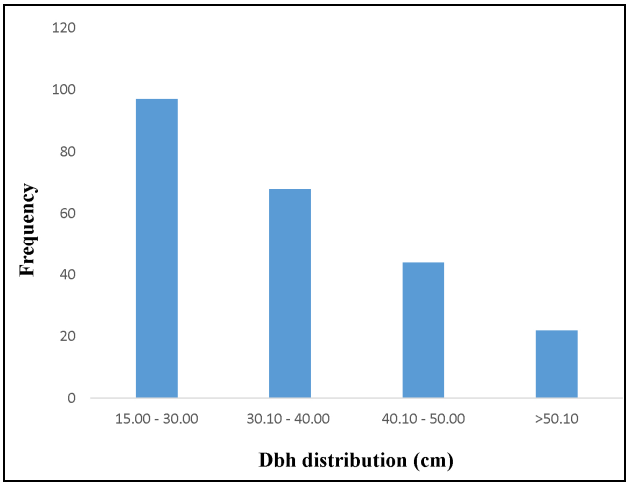

Figure 1a. Stem-diameter distributions of *P. biglobosa* (Jacq) species.

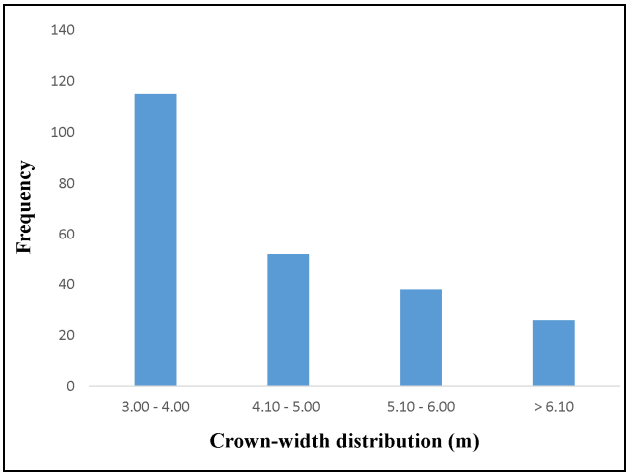

Figure 1b. Crown-diameter distributions of *P. biglobosa* (Jacq) species.

The coefficients of determination differed among the models in this study area.This could be attributed to the fact that almost all the sampled trees were small in sized with relatively small crown diameters (Figures 1a and 1b). These figures show that the tree species are facing exploitation and poor regeneration in the natural forests. The results in Table 1 shows the variation in crown width which correlated with tree diameter (model 3a). The RMSE of the estimates of crown-width was low to make models 3e significantly reliable for predicting crown-width from stem-diameter but the F-ratios and R^2 were very low with negative coefficient in model 3e (-0.0016). The coefficients of the regression were positive and significant different in models 3a and 3b. R^2, RMSE and F-ratio were better in model 3a (Table 1). A comparison of the residual plots (i.e. normal probability plots, histogram and scatter plots) was better on models 3a and 3b than the other models under studied.

Based on the result obtained, the regression analysis results (Table 1 and Figure 2) show that crown width was correlated with bole diameter in model 3a. Thus, linear model (model 3a) was the best model because the model conformed to assumptions of regression analysis with positive coefficients, couple with its superiority in past research [7].

Simple linear model (model 3a) was validated (T-tests), P-values showed no significant difference between the observed (measured crown width) and predicted (crown diameter values) with the Pearson

correlation coefficients of 95%.

This showed a close correlation between the observed and predicted crown diameter values. When the observed data were compared with predicted values using the best model, the T-tests revealed no significant differences (p=0.31 one-tail and 0.63 two-tail).

Growth space requirements for *P. biglobosa* species was determined based on the findings by Foli et al. [7], who reported that growth space was associating with crown size. Thus, using the predicted crown width (Cd=2.244 + 0.137 dbh), the crown area (A) for the species was predicted and expressed in hectare basis (Table 2). For increase production and fast growth of this species, individual tree standshould have unrestricted continuous free-growing space. This requires knowledge of maximum occupancy (stock) of sites. Thus, equations 5 and 6 were used to predict the growing space and stand density for the P. *biglobosa* species.

Table 2 shows that a dominant free-growing tree species of diameter 50.66 cm would require a growing space of 0.0066 ha and stocking of tree stands of 151 trees per hectare. The results from the model for crown-width showed that the model was more elastic between 17.50 and 60.50 cm and gradually decreased as the tree diameter size increased (Table 2).

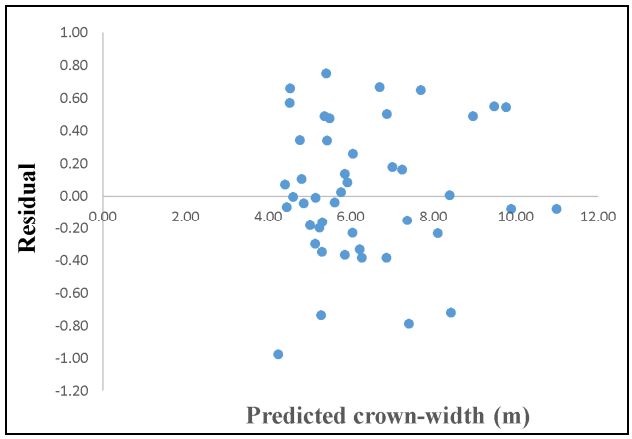

Figure 2. Scatter plot of residuals and predicted crown-diameter for *P. biglobosa* (Jacq) species in the study area.

Table 2. Predicted crown-width (Cd), growth space (S), stocking (N) and stand density (D) using simple linear model (model 3a) in the study area.

Dbh (cm)	Cd (m)	Cd/dbh	S (ha)	Nha^{-1} (1/S)	D (m^2ha^{-1})
17.50	4.64	0.265	0.0017	591	0.0239
20.40	5.04	0.247	0.0020	501	0.0325
30.00	6.35	0.212	0.0032	315	0.0702
35.20	7.07	0.201	0.0039	255	0.0966
37.50	7.38	0.197	0.0043	234	0.1097
45.55	8.48	0.186	0.0057	177	0.1618
50.66	9.18	0.181	0.0066	151	0.2002
60.50	10.53	0.174	0.0087	115	0.2855
70.00	11.83	0.169	0.0110	91	0.3822
75.80	12.63	0.167	0.0125	80	0.4482
80.50	13.27	0.165	0.0138	72	0.5055
90.57	14.65	0.162	0.0169	59	0.6398
100.00	15.94	0.159	0.0200	50	0.7800

The above table was derived from the crown-width and stem-diameter simple linear model, and was computed as follows: Cw = 2.244 + 0.137 dbh; Growing space (S) = Cw^2 π /40 000 and limiting stocking (N) = 1/ S. Where: Cw = crown width, dbh = diameter at breast height.

The crown width and bole diameter ratio is a measure of the efficiency of a tree to accumulate diameter at breast height per unit of crown area. The higher the ratio, the more efficient a tree species is at accumulating dbh [11]. The results on ratio shown that for each meter of crown diameter in *P. biglobosa* species, 0.186 cm of stem diameter was accumulated as the highest efficient without

serious crown interference or competition.

For optimum planting spacing and fat growth, *P. biglobosa* species would require planting spacing of 4 x 4 m. This spacing could lead to reduction of mortality and would increase total production per unit area in a given period of time. Stands with wider spacing in time, have larger average stem and crown diameters than similar stands with closer spacing. The tree species would require low densities (for timber purpose) and high densities (for non-timber purpose). For fast growth and high yield for the purpose of timber, silvicultural practice (such as thinning) has to be apply on the plantation to create more space (8 m x 8 m) i.e. low density; this isto achieve maximum woods for timber production.The result agreed with Clutter et al. [18], who reported that very low densities are required to produce maximum diameter growth throughout the life of an even-aged stand.

4. CONCLUSION

Crown widthcan be predicted from the measurement of tree dbh with a simple linear model. Based on the result obtained, *P. biglobosa* trees would require growth planting spacing of 4 x 4 meters for plantation establishment for the purpose of non-timber forest products, fencing pole and electricity pole. While for timber purpose, silvi-cultural practices (thinning and selection felling) can be applied to the plantation to create more space of 8 x 8 m, for diameter increaseand fast growth.

It is recommended that for sustainable forest and plantations establishment of this species in the study area, crown width can be predicted with simple linear model:

Crown width (Cw) = 2.244 + 0.137 dbh

and growth space can be predicted using the crown-width model (simple linear):

growth space = ($Cw^2\pi$ /4) / 10,000 per hectare

The recommended planting spacing would enhance optimum planting spacing and control competition among the tree species high production and yield.

AUTHOR'S CONTRIBUTION

All the authors contributed equally for the success of this research. The final manuscript has been read and approved by all the authors.

TRANSPARENCY DECLARATION

The authors declare no conflict of interest.

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Research Article

Immunopharmacological activity of flavonoids from *Lemna minor* (Duckweed) and determined its immunological activity

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ABSTRACT

The main objective of our study is to conduct its immunopharmacological activity using flavonoids extracted from whole plant of *Lemna minor* (duckweed), medicinal plant in virally infected human whole blood against ovalbumin (OVA), specific protein antigen. For these studies, isolated secondary metabolite i.e. flavonoid from whole plant of duckweed and evaluated its immuno-pharmacological activity of flavonoid using variable concentration (i.e. 1-30 mg/ml; 50 µl) on infected human whole blood samples and determine its proliferation assay containing OVA and estimation of free haemoglobin content in blood plasma. In addition, antibody production was also estimated against OVA using Elisa method. The results of these studies showed that these flavonoids at higher doses showed immunosuppressive effect because of decline in proliferation, free haemoglobin content in the blood plasma and antibody production. Overall, this study claimed that these flavonoids from duckweed showed immunosuppressive activity against OVA.

Keywords: *Lemna minor*; Ovalbumin; Infected; Proliferation; Haemoglobin.

1. INTRODUCTION

Medicinal plants produce a variety of biologically active compounds that can be subdivided into two categories i.e. primary metabolites and secondary metabolites [1]. These metabolites especially primary that are essential for the survival of the medicinal plant which includes sugars, proteins and amino acids. On the other hand, secondary metabolites showed many uses or medicinal properties relevant to animal and human health, some of them are beneficial and few of them are toxic [1, 2]. The major difference between these two metabolites i.e. primary that directly involved in the growth and development of medicinal plant whereas secondary metabolites are present in the form of compounds that are produced in other metabolic pathways but these are not essential to the functioning of the medicinal plant. In other words, these secondary metabolites from medicinal plant products are also used in signaling and regulation of primary metabolic pathways. In general, these secondary metabolites are present in the form of flavonoids, terpenoids, alkaloids, saponin, glycosides etc. [3, 4].

One of the major components of secondary metabolites i.e. flavonoids and terpenoids that are present almost in every medicinal plant. Most familiar example of terpenoid i.e. artemisinin (malaria) and taxol (cancer) are widely used as medicine and manufactured by various pharmaceutical companies for these diseases [5-7]. All organisms naturally produce some terpenoids as a part of primary metabolism but many produce terpenoids via secondary metabolism [5-7]. Similarly, flavonoids (e.g. quercetin, kaempferol, catechins, and anthocyanidins) is well known for its antioxidant and anti-inflammatory properties [8-10]. In view of these secondary metabolites especially flavonoids that are present abundantly in most of these medicinal plant products and researchers start focusing on these immunopharmacological activities.

One of the aquatic plants i.e. duckweed that freely float on the surface of water. This plant is especially seen in ponds, waste water etc. but is present in the form of dense colonies. These colonies eliminated submerged plants and this could be due to the blockage of light penetration [11, 12]. Most of aquatic birds, snails and fishes consumed duckweed and it will transport to other bodies of water. In general, most of duckweed colonies that are present abundantly on the surface of water which is totally covered and eliminated oxygen level in the water and ultimately it leads to show some harmful or adverse effects related to aquatic animals. Duckweeds (monocotyledonous aquatic plants; family *Araceae*) are one of the world's smallest, fastest ever growing plant and can multiply in a very short period of time [13-15]. In this study, we discussed about its immunopharmacological activity of secondary metabolites especially flavonoids extracted from whole part of duckweed against OVA, specific protein antigen.

2. MATERIALS AND METHODS

2.1. Plant material

Duckweed, whole plant material was collected from VSBT pond, Baramati, Maharashtra. First of all, whole part was washed thoroughly under tap water and then with distilled water. Thereafter, duckweeds were dried in a shady area and grounded into uniform powder using mortar and pestle. The powder was used for extracting secondary metabolites i.e. flavonoids and determined its immunopharmacological activity.

2.2. Extraction of flavonoids

Similarly, qualitative based studies of duckweed powder were done in order to estimate its flavonoid content. For confirmation of flavonoid using lead acetate test, take small amount of lead acetate solution is added into the duckweed powder and yellow colour precipitation will appear, it indicates the presence of flavonoid content.

In quantitative based studies, duckweed powder (1 g) was dissolved in methanol (80%, 10 ml) and then warm for 2 h at 100ºC. Afterwards, cool down the solution and then collect the filtrate using Whatman filter paper and add ethyl acetate (10 ml) along with distilled water (20 ml), shaking regularly for at least 5 minutes and then incubate the solution overnight at room temperature. After incubation, two different layers were observed i.e. upper layer (i.e. ethyl acetate) and lower layer (flavonoids). Finally, evaporate the upper phase i.e. ethyl acetate solution and then dried the plant extracts (flavonoids) settled at the bottom [16].

2.3. Lymphocyte proliferation assay

Anti-coagulant, EDTA human blood samples (virally infected) were collected from Mangal Pathology Laboratory, Baramati, Maharashtra, India. In this study, lysed human whole blood (100 µl) were cultured with variable doses of flavonoids (1-30 mg/ml, 50 µl) extracted from duckweed powder along with or without ovalbumin (1 mg/ml, 50 µl). Incubate 96-well plates for 48 h at 37°C. OVA used as standard for these immunological studies. Centrifuging (2500 rpm for 10 minutes at 4°C) the plates and then add fresh complete medium was added into the 96-well plates. Again, incubating the plates for another 4 h along with MTT (5 mg/ml, 10 µl) continued. After incubation, the plates were suddenly centrifuged with discarding the supernatant, collecting the pellet and finally dispersing in dimethyl sulphoxide (DMSO) solution. The optical density was measured at 570 nm [17, 18].

2.4. Estimation of free haemoglobin

Lysed virally infected human whole blood (n = 6; 10^5 cells/well; 100 µl) were collected and cultured in 96 well flat bottom tissue culture plate for 48 h incubation along with variable doses of flavonoids (1-30 mg/ml; 50 µl). Collect and transfer the samples from culture plate into 3 ml falcon tube. Centrifuge the samples at 6000 rpm at 4ºC and then washed with PBS pertaining to observe the free haemoglobin in the supernatant. Finally samples were analysed through UV visible spectrophotometer at 570 nm [19].

2.5. ELISA

Indirect Elisa was performed for estimating antibody production against ovalbumin (OVA, 100 µg/well) using variable doses of flavonoids and terpenoids (1-30 mg/ml; 50 µl). OVA used as coating antigen and incubate the plate for overnight at 4ºC. After incubation, first of all block this plate with 1% bovine serum albumin (BSA). Incubate the plate for one hour at room temperature and then wash the plate with PBS (2-3 times). Thereafter, add variable concentration of flavonoids and terpenoids (1-30 mg/ml; in 96 well plate. Incubate the plate for another 4 h incubation at carbon dioxide incubator. Afterwards, again wash the plate with PBS (2-3 times) and then add secondary antibody (horse antiserum; 1:10000 dilution). Incubate the plate for another 1h at carbon dioxide incubator. After incubation, wash the plate with OBS and then add substrate, TMB. Incubate the plate for another 10-15 minutes in dark at room temperature. Afterwards, stop solution was added and optical density was measured at 450 nm [20].

2.6. Statistical analysis

The difference between control and treated group of flavonoids extracted from duckweed is determined by one way ANOVA test (Bonferroni multiple comparison test). *P <0.05; *P<0.01; ***P<0.001.

3. RESULTS

3.1. Lymphocyte proliferation assay

The effect of variable doses of flavonoids from duckweed on antigen (OVA) specific immune response in virally infected lysed human whole blood as shown in Fig. 1. The results showed its decline in proliferation rate at higher doses of flavonoids and terpenoids. Overall, this study showed its immunosuppressive effect.

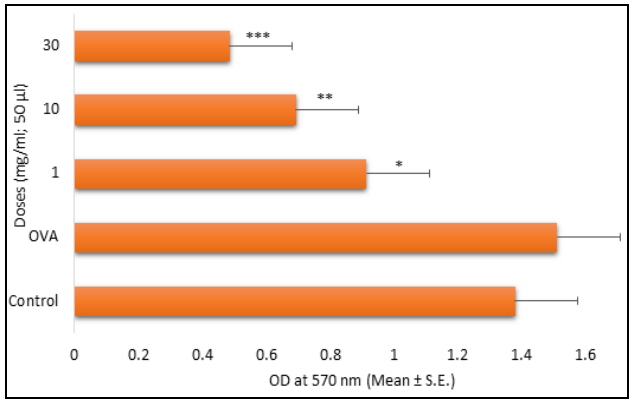

Figure 1. Proliferation assay. To determine the effect of variable doses of flavonoids (1-30 mg/ml, 50 µl) extracted from whole plant of duckweed on virally infected lysed human whole blood. Values are expressed as Mean ± S.E. The difference between the controls versus variable doses of flavonoid is determined by one way ANOVA test (Bonferroni multiple comparison test).).*P<0.05; **P<0.01, ***P<0.001.

3.2. Estimation of free haemoglobin

At higher doses of flavonoids showed decline in free haemoglobin content as shown in Fig. 2 in virally infected lysed human whole blood as compared to control.

3.3. ELISA

The results of these studies related to flavonoids on antibody production against OVA as shown in Fig. 3. At lower doses, there is slightly enhancement in antibody production but at higher doses, there is decline in antibody production as compared to control.

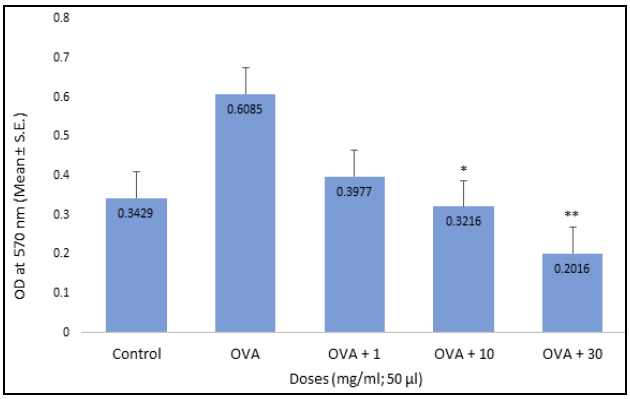

Figure 2. Estimation of free haemoglobin content. To determine the effect of variable doses of flavonoids (1-30 mg/ml, 50 µl) extracted from whole plant of duckweed on total haemoglobin content in virally infected lysed human whole blood. Values are expressed as Mean ± S.E. The difference between the controls versus variable doses of flavonoid is determined by one way ANOVA test (Bonferroni multiple comparison test). *P<0.05; **P<0.01, ***P<0.001.

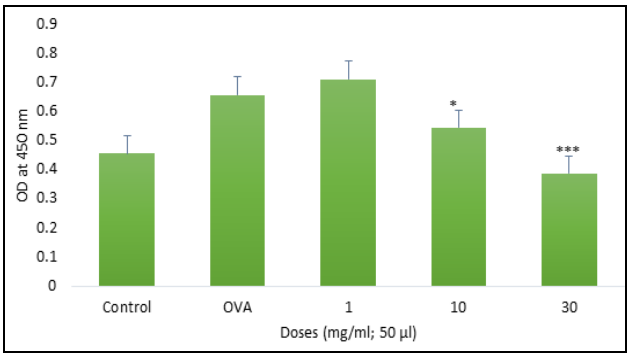

Figure 3. ELISA. Indirect ELISA was assayed using OVA as coating antigen using variable doses of flavonoid from whole plant of duckweed for determining antibody titre. Horse anti-serum used as secondary antibody. The difference between control and variable doses of flavonoid is determined through one way ANOVA test (Bonferroni multiple comparison test). *P < 0.05; **P < 0.01 and ***P < 0.001.

4. DISCUSSION

The use of various medicinal plant products is believed to be an age-old tradition. Numerous studies were conducted by various researchers and proved that these natural plant based products are useful for diverse ailments and diseases. Most of the health care professionals including researchers all over the world have shown some interests in the medicinal uses of these plant products but lot of confusion related to its identification, effectiveness, efficacy, dosage, toxicity, standardization, and regulation. As per WHO, traditional medicine is more popular in all regions of the world and its use is rapidly expanding even in developed countries as well. In view of this, variety of medicinal plant products that are reported in Baramati region, Maharashtra, India and its knowledge about its medicinal properties has been accumulated regarding various diseases e.g. rheumatoid arthritis, cancer, autoimmune diseases etc. Recently, more than two thousand medicinal plants are mentioned in Ayurvedic systems of medicine [1]. Out of these, number of medicinal plants that are reported and claimed its immunosuppressive properties. In view of this, we worked on various medicinal plants especially *Lemna minor* (duckweed) [11, 12] and extracted secondary metabolites i.e. flavonoids and determined its immunological activity against specific protein antigen. In this study, our results showed that these flavonoids showed immuno-suppressive effect in case of virally infected human whole blood samples at higher doses. This activity of this fraction especially flavonoids and terpenoids may be attributed due to the presence of active molecules in the extract [9, 10]. The capacity of these active molecules may have some useful applications in various disease disorders e.g. autoimmune disease, organ transplant rejection etc.

In this study, exposure of variable doses of flavonoids isolated from duckweed, medicinal plant products caused a reduction in free haemoglobin content at higher doses in case of lysed virally infected human whole blood samples. Due to sudden decline in free haemoglobin content in virally infected blood clearly showed its immunosuppressive effect. Further immunological studies are needed in order to confirm its immunosuppressive activity of this medicinal plant and evaluate its activity or potential in the treatment of various disorders. According to the literature, major factors that are associated with virally infected blood profile i.e. high haemoglobin concentration in blood plasma. Regulation of these components using flavonoids, secondary metabolite from duckweed, medicinal plant is the major goal of this study.

Immunological validation of these flavonoids isolated from duckweed and proved its efficacy in

order to reducing the free haemoglobin content in virally infected blood and also reduction in antibody production against specific protein antigen. From these results related to its potential effectiveness against virally infected blood samples, it is assumed that these flavonoids isolated from medicinal plant products that played in the management of infectious diseases, which needs further exploration for necessary development of drugs and nutra-ceuticals from natural resource.

5. CONCLUSION

This study showed its immunosuppressive effect of flavonoid from duckweed against specific protein antigen. Further immunological investi-gation is also required to extract the active compound which can be observed as a potent immunosuppressive drug.

AUTHOR'S CONTRIBUTION

This work was carried out in collaboration between three authors. SS, AG and VM designed the study, wrote the protocol and interpreted the data where SS and AG anchored the field study, gathered the initial data related to his M.Sc. Microbiology dissertation work under AG guidance and performed preliminary data analysis. AG, SS, BS and VM managed the literature searches whereas AG and SS produced the initial draft. The final manuscript has been read and approved by all authors.

TRANSPARENCY DECLARATION

Authors have declared that no conflict of interests exists.

ETHICAL APPROVAL

These studies were conducted under IBSC guidelines and approved by Savitribai Phule Pune University.

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