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# Supplementary file 1

**Table S1.** Search strategy for databases

| Database | Search Strategy |
|---|---|
| **PubMed** | ((((idiopathic proctocolitis) OR (colitis gravis)) OR (inflammatory bowel disease*)) OR ((colitis) AND (ulcerative))) AND ((spirulina) OR (arthrospira)) |
| **Scopus** | ( ( ( ALL ( idiopathic AND proctocolitis ) OR ALL ( colitis AND gravis ) OR ALL ( inflammatory AND bowel AND disease* ) ) ) OR ( ( ALL ( colitis ) AND ALL ( ulcerative ) ) ) ) AND ( ( ALL ( spirulina ) OR ALL ( arthrospira ) ) ) |
| **Web of Science** | ( ( ALL =( idiopathic proctocolitis ) OR ALL =( colitis gravis ) OR ALL =( inflammatory bowel disease* ) OR ( ( ALL =( colitis ) AND ALL= ( ulcerative ) ) ) ) AND ( ALL= ( spirulina ) OR ALL= ( arthrospira ) )) |
| **Embase** | ((idiopathic AND proctocolitis) OR (colitis AND gravis) OR (inflammatory AND bowel AND disease*) OR (ulcerative AND colitis)) AND (spirulina OR arthrospira) |

**Table S2.** Inclusion and exclusion criteria

| PICOS | Inclusion Criteria | Exclusion Criteria |
|---|---|---|
| **Population** | Animal models (mammalian) of ulcerative colitis or patients with this disease | Other types of inflammatory bowel diseases or other animals |
| **Intervention** | Spirulina (*Arthrospira platensis, A. fusiformis, and A. maxima.*) or its extracts, alone or in combination of routine treatment | Combination of spirulina with other agents or synthetic forms; Components of spirulina instead of whole algae |
| **Comparator** | Placebo, routine treatment | No control group |
| **Outcome** | The primary outcome was disease activity, and the secondary outcomes were histopathological changes, inflammatory or oxidative markers, microbiota composition, and anthropometric and psychological features | Irrelevant outcomes or those that were not reported as absolute values |
| **Study design** | Experimental animal or human studies | Review, Note, Editorial, letter, Observational studies, Quasi-experimental studies, in vitro studies |

**Table S3.** The risk of bias assessment and tier classifications of the included animal studies on the effects of spirulina supplementation on ulcerative colitis

| CATEGORY | QUESTIONS | Arrari F, 2024[16] | Zhong D, 2024[24] | Wang N, 2024[23] | Chen QW[27] | Wang J, 2022[22] | Yacoub MB, 2022[17] | Burkhardt W, 2021[26] | Zhong D, 2021[25] | Garcia FAO, 2020[28] | Guo W, 2019[29] | Morsy MA, 2019[9] | Ghazy EW, 2019[20] | Rezaei N, 2019[10] | Rezaei N, 2018[21] | Abdel-Daim MM, 2015[18] | Coskun ZK, 2011[19] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Selection Bias | 1. Was administered dose or exposure level adequately randomized? | - | + | + | + | + | + | - | + | + | + | + | + | + | - | - | + |
| | 2. Was allocation to study groups adequately concealed? | - | + | + | + | + | + | - | + | + | + | + | + | + | - | - | + |
| Performance Bias | 3. Were experimental conditions identical across study groups? | ++ | ++ | ++ | ++ | - | - - | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | - |
| | 4. Were the research personnel and human subjects blinded to the study group during the study? | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| Attrition/ Exclusion Bias | 5. Were outcome data complete without attrition or exclusion from analysis? | ++ | + | ++ | + | + | ++ | + | ++ | + | ++ | ++ | + | + | ++ | + | ++ |
| Detection Bias | 6. Can we be confident in the exposure characterization? | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | - | ++ | ++ | ++ | ++ | ++ | ++ | - |
| | 7. Can we be confident in the outcome assessment? | - | - | - | - | ++ | - | ++ | - | ++ | ++ | - | - | ++ | ++ | ++ | ++ |
| Selective Reporting Bias | 8. Were all measured outcomes reported? | ++ | + | ++ | ++ | + | ++ | + | ++ | ++ | ++ | ++ | ++ | ++ | - | ++ | ++ |
| Other Sources of Bias | 9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)? | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Overall Tier | | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 2 |

++ Definitely low risk of bias

\+ Probably low risk of bias

\- Probably high risk of bias

\- - Definitely high risk of bias

Tier 1 = low risk, Tier 2 = moderate, Tier 3 = high risk of bias

**Table S4.** The risk of bias assessment and tier classifications of the included clinical trials on the effects of spirulina supplementation on ulcerative colitis

| CATEGORY | QUESTIONS | Moradi S, 2024[11] | Moradi S, 2023[30] | Moradi S, 2023[31] |
|---|---|---|---|---|
| Selection Bias | Random sequence generation | + | + | + |
| | Allocation concealment | ? | ? | ? |
| Performance Bias | Blinding of participants and personnel | + | + | + |
| Attrition Bias | Incomplete outcome data addressed | + | + | + |
| Detection Bias | Blinding of outcome assessment | + | + | + |
| Reporting Bias | Selective reporting | - | + | + |
| Other Sources of Bias | Anything else | + | + | + |
| Overall Tier | | | | |

+ Low risk of bias
? Unclear risk of bias
- High risk of bias

**Table S5.** Stratified meta-analysis of the effects of spirulina on clinical and histopathological features in ulcerative colitis

| Variable | | Subgroups | Number of effect sizes | Pooled effect size (95% CI) | P-for-difference | P-for-heterogeneity | $I^2$ (%) |
|---|---|---|---|---|---|---|---|
| **Weight change** | All | | 19 | 8.61 (2.98 to 14.25) | <0.001 | <0.001 | 99.78 |
| | Type of animals | *Rat* | 9 | 14.35 (3.52 to 25.18) | 0.04 | < 0.001 | 97.84 |
| | | *Mice* | 10 | 2.90 (1.90 to 3.91) | | < 0.001 | 80.65 |
| | Spirulina form | *Whole* | 13 | 3.79 (0.68 to 6.90) | 0.05 | < 0.001 | 98.58 |
| | | *Extract* | 6 | 17.91 (4.29 to 31.53) | | < 0.001 | 99.70 |
| | UC model | *AA* | 5 | 11.07 (-2.95 to 25.10) | 0.11 | < 0.001 | 96.24 |
| | | *DSS* | 10 | 8.25 (0.22 to 16.28) | | < 0.001 | 99.82 |
| | | *Others* | 4 | 1.54 (1.20 to 1.88) | | 0.29 | 0.00 |
| | ROB | *Low risk* | 15 | 9.97 (3.51 to 16.43) | 0.33 | < 0.001 | 99.72 |
| | | *Moderate risk* | 4 | 3.33 (-8.33 to 14.99) | | < 0.001 | 91.59 |
| **Disease activity index** | All | | 10 | -2.39 (-2.95 to -1.83) | <0.001 | 0.21 | 5.89 |
| | Type of animals | *Rat* | 6 | -2.37 (-3.04 to -1.70) | 0.61 | 0.49 | 0.00 |
| | | *Mice* | 4 | -2.83 (-4.49 to -1.18) | | 0.05 | 63.96 |
| | Spirulina form | *Whole* | 6 | -2.55 (-3.26 to -1.84) | 0.80 | 0.45 | 0.00 |
| | | *Extract* | 4 | -2.36 (-3.63 to -1.09) | | 0.08 | 51.23 |
| | ROB | *Low risk* | 8 | -2.19 (-2.77 to -1.60) | 0.09 | 0.24 | 0.00 |
| | | *Moderate risk* | 2 | -3.59 (-5.08 to -2.09) | | 0.93 | 0.00 |
| **Colon length** | All | | 11 | 1.25 (0.59 to 1.91) | <0.001 | <0.001 | 95.80 |
| | Type of animals | *Rat* | 2 | 2.74 (0.80 to 4.69) | 0.09 | 0.01 | 84.08 |
| | | *Mice* | 9 | 0.97 (0.40 to 1.54) | | < 0.001 | 94.32 |
| | Spirulina form | *Whole* | 7 | 0.78 (0.59 to 0.98) | 0.14 | 0.67 | 0.00 |
| | | *Extract* | 4 | 1.99 (0.40 to 3.59) | | < 0.001 | 98.69 |
| **Histopathological score** | All | | 9 | -1.89 (-4.51 to 0.73) | 0.16 | <0.001 | 94.31 |
| | Type of animals | *Rat* | 2 | -2.00 (-3.07 to -0.94) | 0.97 | 0.44 | 0.00 |
| | | *Mice* | 7 | -1.93 (-5.62 to 1.76) | | <0.001 | 95.61 |
| | Spirulina form | *Whole* | 7 | -1.22 (-4.04 to 1.60) | 0.38 | <0.001 | 93.79 |
| | | *Extract* | 2 | -5.56 (-14.91 to 3.79) | | 0.01 | 86.15 |
| | ROB | *Low risk* | 7 | -1.93 (-5.62 to 1.76) | 0.97 | <0.001 | 95.61 |
| | | *Moderate risk* | 2 | -2.00 (-3.07 to -0.94) | | 0.44 | 0.00 |

Abbreviations: AA: acetic acid; CI: confidence Interval; DSS: dextran sulfate sodium; ROB: risk of bias; UC: ulcerative colitis

**Table S6.** Stratified meta-analysis of the effects of spirulina on oxidative stress in ulcerative colitis

| Variable | | Subgroups | Number of effect sizes | Pooled effect size (95% CI) | P-for-difference | P-for-heterogeneity | $I^2$ (%) |
|---|---|---|---|---|---|---|---|
| **Colonic MDA** | All | | 8 | -4.47 (-6.52 to -2.42) | <0.001 | <0.001 | 82.62 |
| | Spirulina form | *Whole* | 6 | -3.54 (-5.42 to -1.65) | **0.04** | <0.001 | 79.50 |
| | | *Extract* | 2 | -7.81 (-11.48 to -4.14) | | 0.28 | 13.55 |
| | ROB | *Low risk* | 4 | -6.75 (-10.79 to -2.72) | 0.11 | <0.001 | 76.80 |
| | | *Moderate risk* | 4 | -3.05 (-5.03 to -1.06) | | <0.001 | 74.88 |
| **Colonic SOD** | All | | 4 | 4.02 (2.77 to 5.28) | <0.001 | 0.56 | 0.00 |
| | Spirulina form | *Whole* | 2 | 4.08 (2.36 to 5.81) | 0.81 | 0.98 | 0.00 |
| | | *Extract* | 2 | 4.54 (1.28 to 7.81) | | 0.15 | 50.79 |
| | ROB | *Low risk* | 2 | 4.54 (1.28 to 7.81) | 0.81 | 0.15 | 0.00 |
| | | *Moderate risk* | 2 | 4.08 (2.36 to 5.81) | | 0.98 | 50.79 |
| **Colonic catalase** | All | | 6 | 2.40 (0.15 to 4.66) | 0.04 | <0.001 | 90.50 |
| | Spirulina form | *Whole* | 4 | 1.43 (-0.72 to 3.58) | 0.27 | <0.001 | 89.66 |
| | | *Extract* | 2 | 8.06 (-3.45 to 19.57) | | 0.01 | 83.38 |
| | ROB | *Low risk* | 3 | 4.94 (-2.10 to 11.98) | 0.42 | <0.001 | 96.65 |
| | | *Moderate risk* | 3 | 1.76 (-1.44 to 4.97) | | <0.001 | 91.82 |

Abbreviations: CI: confidence Interval; MDA: malondialdehyde; ROB: risk of bias; SOD: superoxide dismutase; UC: ulcerative colitis

**Table S7.** Stratified meta-analysis of the effects of spirulina on inflammatory markers in ulcerative colitis

| Variable | | Subgroups | Number of effect sizes | Pooled effect size (95% CI) | P-for-difference | P-for-heterogeneity | $I^2$ (%) |
|---|---|---|---|---|---|---|---|
| Colonic TNF-α | All | | 8 | -4.78 (-6.47 to -3.10) | <0.001 | 0.01 | 60.41 |
| | Type of animals | *Rat* | 3 | -5.13 (-8.21 to -2.05) | 0.86 | 0.05 | 67.27 |
| | | *Mice* | 5 | -4.78 (-7.12 to -2.44) | | 0.03 | 59.85 |
| | Spirulina form | *Whole* | 4 | -5.26 (-7.94 to -2.58) | 0.74 | 0.10 | 51.46 |
| | | *Extract* | 4 | -4.62 (-7.141 to -2.11) | | 0.02 | 69.00 |
| | UC model | *AA* | 2 | -3.89 (-6.28 to -1.50) | 0.28 | 0.17 | 46.69 |
| | | *DSS* | 4 | -4.87 (-7.79 to -1.96) | | 0.02 | 71.28 |
| | | *Others* | 2 | -7.21 (-10.54 to -3.88) | | 0.37 | 0.00 |
| | ROB | *Low risk* | 6 | -4.75 (-6.64 to -2.85) | 0.83 | 0.03 | 53.61 |
| | | *Moderate risk* | 2 | -5.41 (-11.06 to 0.23) | | 0.02 | 80.28 |
| Colonic IL-6 | All | | 7 | -4.56 (-7.35 to -1.77) | <0.001 | <0.001 | 88.18 |
| | Type of animals | *Rat* | 2 | -5.49 (-13.48 to 2.05) | 0.80 | <0.001 | 88.14 |
| | | *Mice* | 5 | -4.38 (-7.64 to -1.12) | | <0.001 | 81.63 |
| | Spirulina form | *Whole* | 3 | -3.78(-6.78 to -0.77) | 0.61 | 0.08 | 58.45 |
| | | *Extract* | 4 | -5.22 (-9.96 to -0.48) | | <0.001 | 89.45 |
| Colonic IL-1β | All | | 5 | -5.81 (-8.88 to -2.75) | <0.001 | <0.001 | 76.86 |
| | Type of animals | *Rat* | 3 | -6.13 (-11.53 to -0.73) | 0.92 | <0.001 | 84.81 |
| | | *Mice* | 2 | -5.83 (-8.28 to -3.37) | | 0.63 | 0.00 |
| | Spirulina form | *Whole* | 2 | -4.49 (-10.91 to 1.92) | 0.54 | <0.001 | 87.41 |
| | | *Extract* | 3 | -6.62 (-8.961 to -4.28) | | 0.32 | 5.95 |
| | ROB | *Low risk* | 3 | -6.62 (-8.961 to -4.28) | 0.54 | 0.32 | 5.95 |
| | | *Moderate risk* | 2 | -4.49 (-10.91 to 1.92) | | <0.001 | 87.41 |
| Colonic MPO | All | | 8 | -2.79 (-4.29 to -1.29) | <0.001 | <0.001 | 83.48 |
| | Type of animals | *Rat* | 5 | -3.19 (-4.60 to -1.77) | 0.93 | 0.04 | 61.99 |
| | | *Mice* | 3 | -3.46 (-9.54 to 2.61) | | 0.01 | 97.93 |
| | Spirulina form | *Whole* | 2 | -2.71 (-3.93 to -1.49) | 0.71 | 0.85 | 0.00 |
| | | *Extract* | 6 | -3.22 (-5.67 to -0.77) | | <0.001 | 91.77 |
| | UC model | *AA* | 3 | -3.00 (-4.09 to -1.92) | 0.88 | 0.58 | 0.00 |
| | | *DSS* | 5 | -3.26 (-6.44 to -0.07) | | <0.001 | 94.74 |
| Serum TNF-α | All | | 6 | -2.85 (-3.93 to -1.78) | <0.001 | 0.03 | 43.16 |
| | Type of animals | *Rat* | 4 | -2.65 (-3.84 to -1.46) | 0.39 | 0.10 | 50.39 |
| | | *Mice* | 2 | -6.18 (-14.14 to 1.78) | | 0.02 | 80.11 |
| | Spirulina form | *Whole* | 3 | -2.03 (-2.91 to -1.14) | 0.08 | 0.74 | 0.00 |
| | | *Extract* | 3 | -5.08 (-8.35 to -1.82) | | 0.05 | 71.73 |
| | UC model | *AA* | 2 | -1.86 (-2.84 to -0.88) | 0.04 | 0.90 | 0.00 |
| | | *DSS* | 4 | -3.86 (-5.46 to -2.26) | | 0.07 | 37.95 |
| Serum IL-6 | All | | 6 | -8.66 (-16.03 to -1.29) | 0.02 | <0.001 | 97.65 |
| | Type of animals | *Rat* | 4 | -7.70 (-15.61 to 0.20) | 0.66 | <0.001 | 97.02 |
| | | *Mice* | 2 | -13.77 (-39.30 to 11.76) | | <0.001 | 88.10 |
| | Spirulina form | *Whole* | 3 | -3.44 (-5.41 to -1.47) | 0.11 | 0.11 | 56.29 |
| | | *Extract* | 3 | -16.39 (-32.33 to -0.44) | | <0.001 | 89.86 |
| | UC model | *AA* | 2 | -4.43 (-7.50 to -1.36) | 0.24 | 0.11 | 59.88 |
| | | *DSS* | 4 | -12.20 (-24.88 to 0.47) | | <0.001 | 98.52 |
| Serum IL-1β | All | | 4 | -5.01 (-8.78 to -1.23) | 0.01 | <0.001 | 85.39 |
| | Type of animals | *Rat* | 2 | -5.02 (-7.72 to -2.32) | 0.84 | 0.20 | 39.26 |
| | | *Mice* | 2 | -6.14 (-16.89 to 4.60) | | 0.01 | 86.82 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | UC model | *AA* | 2 | -5.02 (-7.72 to -2.32) | 0.84 | 0.20 | 39.26 |
| | | *DSS* | 2 | -6.14 (-16.89 to 4.60) | | 0.01 | 86.82 |

Abbreviations: AA: acetic acid; CI: confidence Interval; DSS: dextran sulfate sodium; IL: interleukin; MPO: myeloperoxidase; ROB: risk of bias; TNF-α: tumor necrosis factor-alpha; UC: ulcerative colitis

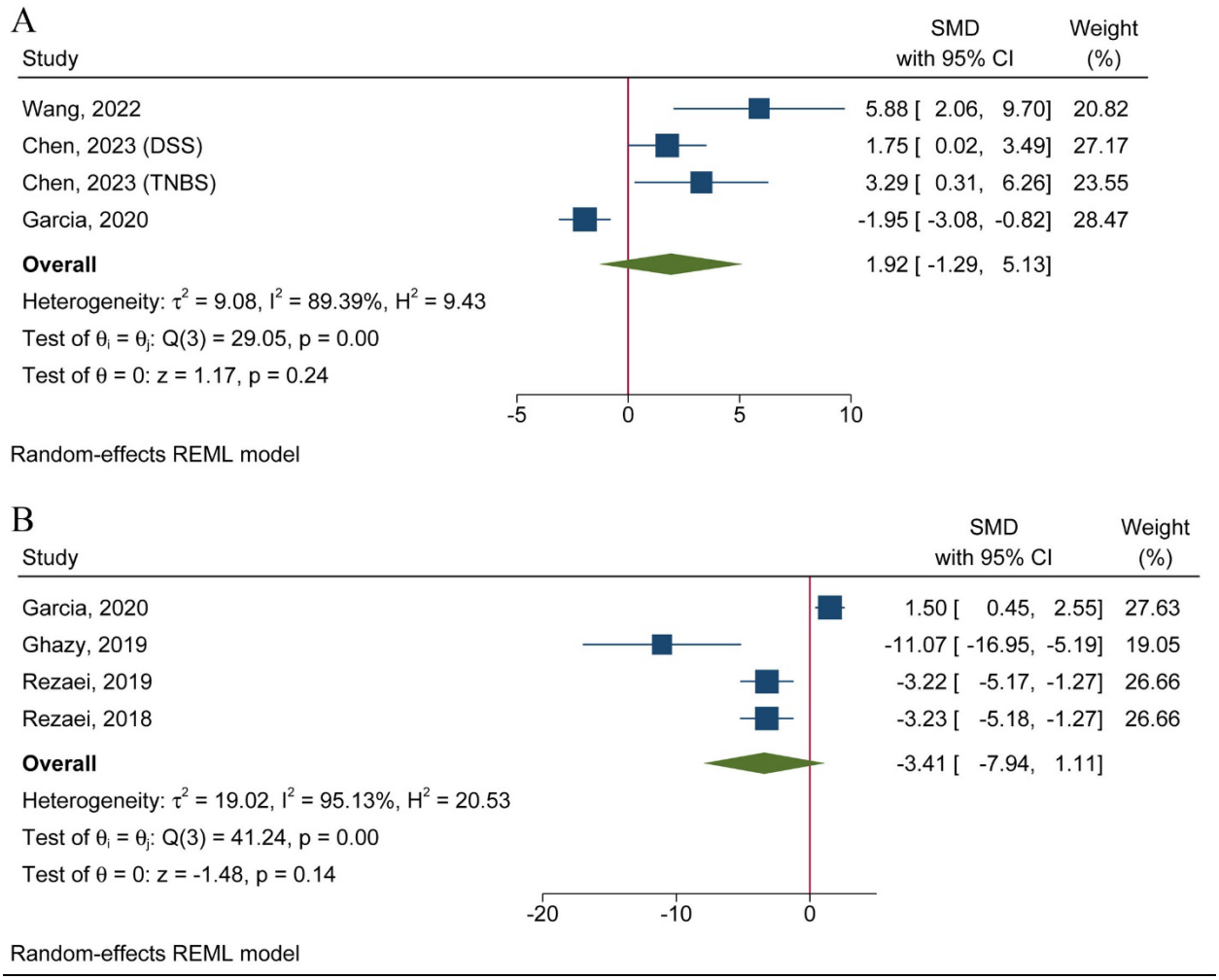


Figure S1. Forest plot depicting the effects of spirulina on colonic interleukin-10 (A) and nitric oxide (B) in ulcerative colitis

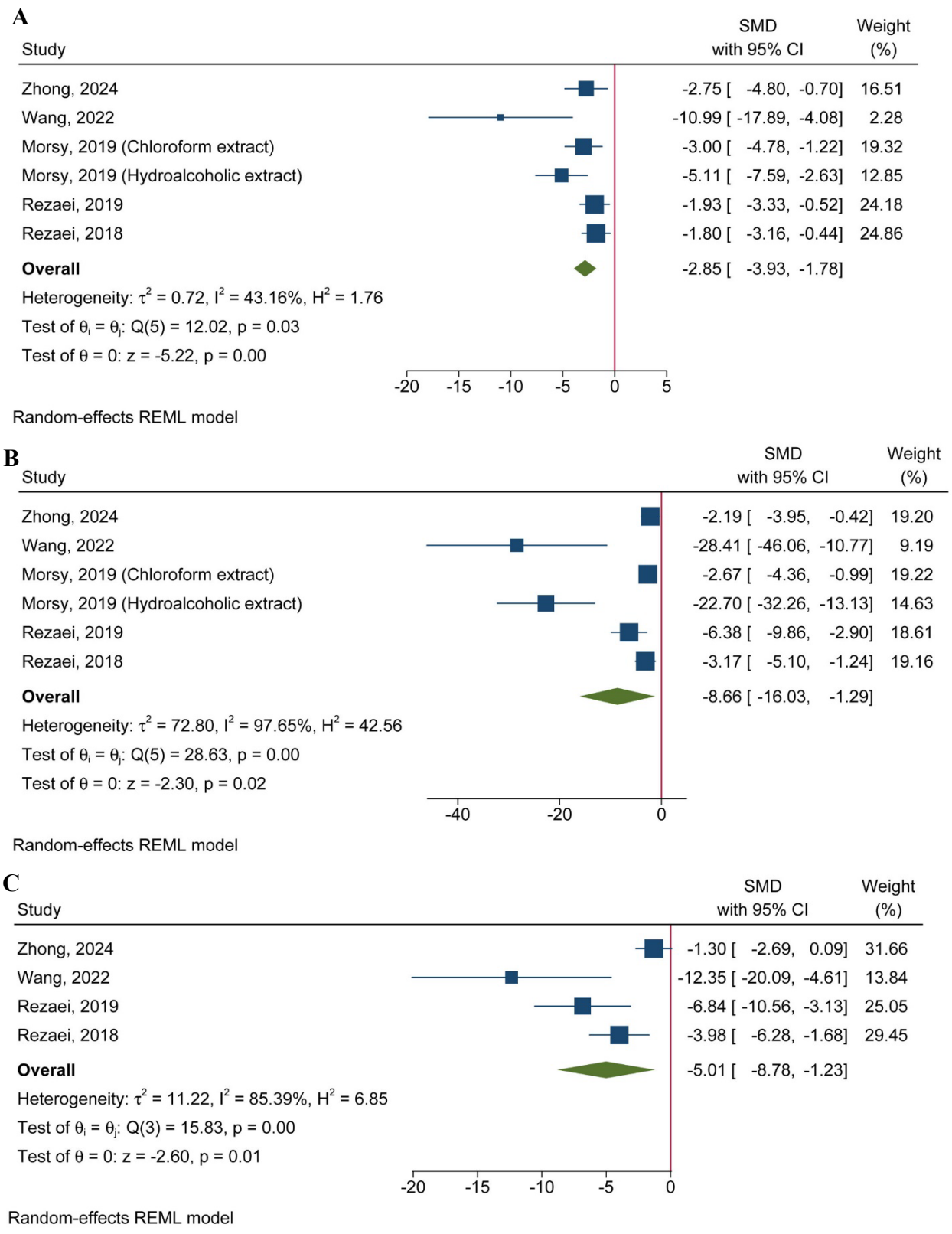


Figure S2. Forest plot depicting the effects of spirulina on the serum levels of inflammatory markers: tumor necrosis factor-α (A), interleukin-6 (B), and interleukin-1β (C) in ulcerative colitis

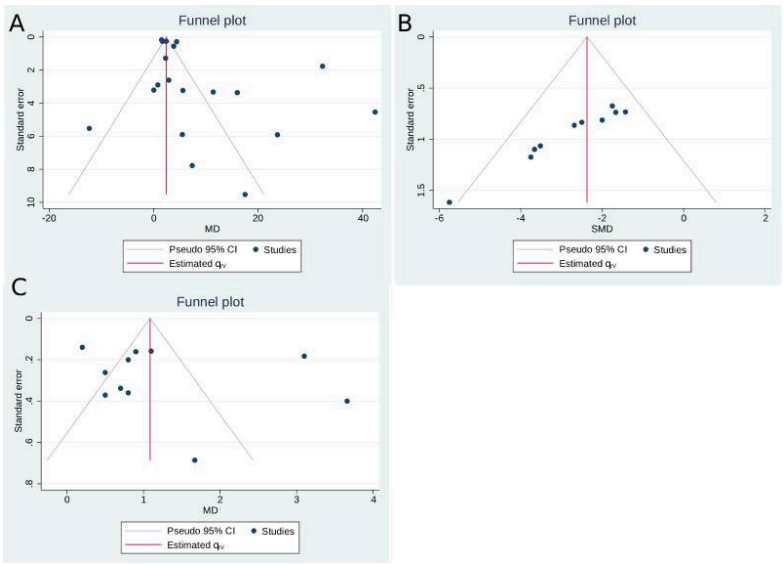


Figure S3. Funnel plots for the effects of spirulina on body weight (A), disease activity index (B), and colon length (C) in ulcerative colitis

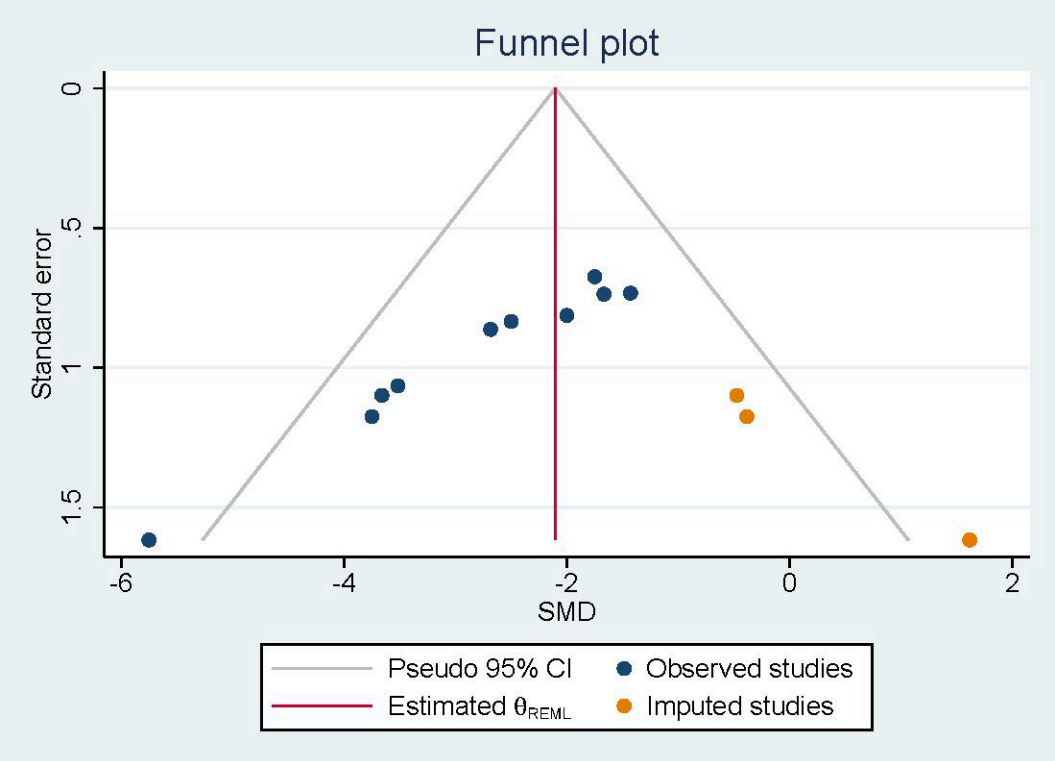


Figure S4. Trim and fill analysis for the effects of spirulina on disease activity index in ulcerative colitis